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Excess Electron Transfer from an Internally Conjugated Aromatic Amine to 5-Bromo-2′-deoxyuridine in DNA

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Electron transfer in DNA has received considerable attention during the past decade[1] and is now widely explored through both theoretical and practical approaches to evaluate its importance in DNA damage and repair as well as for application to nanoscale devices.[2] Especially, oxidative electron transfer (hole transfer) in DNA is implicated in radiation-induced DNA base damage[3,4] and also in nucleobase oxidation by transition metals,[5] such as $CoCl_2$ with HSO_5^-,[6] because the site of oxidation can migrate from one nucleotide sequence to another with a lower oxidation potential.[7] Intensive study of excess electron migration through DNA in contrast has only begun in the last several years.[4,8,9] Inspired by photoenzymatic repair of thymine cyclobutane dimer in DNA,[10] Carell and co-workers have synthesized duplex DNA containing a flavin and a thymine dimer, and with this they successfully observed a weak distance dependence of excess electron transfer at ambient temperature.[8]

For structure−activity studies on excess electron transfer, our laboratory has now developed a convenient alternative to existing systems by avoiding the need to synthesize phosphoramidite derived electron donors and acceptors. As reported below, electron transfer from an internally conjugated aromatic amine to 5-bromo-2′-deoxyuridine (BrdU) can be readily detected because subsequent formation of a uridine-5-yl radical derived from BrdU induces spontaneous and alkaline-dependent cleavage of the nucleic acid strand.[11,12]

Aromatic amines are often used as photoinduced electron donors due to their low excited state oxidation potentials and easily accessible excitation bands above 300 nm.[13] For example, *N*,*N*-dimethylaniline has been used to model the reductive, light-dependent mechanism of thymine dimer repair in nature.[14] Our chosen electron donor, an analogue of *N*,*N*,*N*′,*N*′-tetramethyl-1,5-diaminonaphthalene (TMDN), is a powerful reductant ($E^*_{ox} \approx -2.8$ V vs SCE) and has a low energy excited state ($\lambda_{max} = 325$ nm), which enables selective excitation of the chromophore without direct excitation of DNA bases.[15] Furthermore, the planar aromatic ring might allow for intercalation within DNA, leading to efficient electron transfer due to orbital overlap between nucleobases and the chromophore.[1] The desired oligodeoxynucleotide conjugate of the electron donor (ODN 2′) (Chart 1) was prepared by coupling an aminooxy-derivatized TMDN analogue to the aldehyde of an abasic site (Supporting Information).[16] The TMDN analogue was synthesized from 1,5-diaminonaphthalene in four steps (55% total yield), and the abasic site was synthesized from treatment of an oligodeoxynucleotide (ODN 3′, commercially available) containing a triol residue with $NaIO_4$ (Supporting Information).[17]

The effect of excess electron transfer from the donor of ODN 2′ to the BrdU acceptor of ODN 3 was observed after photoirradiation ($\lambda > 335$ nm, under N_2) by strand cleavage at the thymine (T_9) adjacent to BrdU. Direct cleavage accumulated with low efficiency during irradiation (Figure 1a, lanes 1−7), and most cleavage required subsequent treatment with hot alkali (piperidine) (Figure 1a, lanes 8−14) as expected for C1′ oxidation of T_9 by the intermediate uridine-5-yl radical.[11,12] Strand cleavage due to direct excitation of BrdU is negligible under these experimental conditions because no reaction occurred in an equivalent duplex lacking the aromatic amine donor (ODN 3/ODN 1′). Likewise, the donor containing oligodeoxynucleotide did not induce specific strand cleavage at T_9 in the absence of an adjacent BrdU (ODN 1/ODN 2′) (Supporting Information).

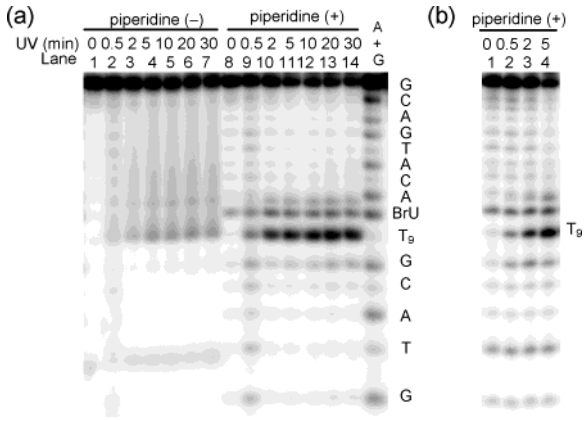
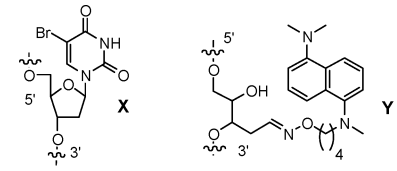


Figure 1. Autoradiograms of 20% denaturing polyacrylamide gels showing strand cleavage as a result of excess electron transfer from a conjugated donor. 5′-^{32}P-labeled ODN 3 (90 nCi, 100 nM) and ODN 2′ (160 nM) were annealed in sodium phosphate (10 mM, pH 7.0) and NaCl (90 mM) and then photoirradiated under N_2 (10 °C) with a high-pressure Xe-arc (1000 W) using a 335-nm cutoff filter. (a) Anaerobic photolysis. Samples were photoirradiated for the indicated periods and either analyzed directly (lanes 1−7) or after subsequent treatment with piperidine at 90 °C for 30 min (lanes 8−14). (b) Anaerobic photolysis in the presence of mannitol (0.2 mM) and N_2O (sat.). Samples were photoirradiated for the indicated periods and treated with piperidine as described above.

Chart 1. Oligodeoxynucleotide Sequences



ODN	sequence
1	5′- GCA GTA CGT TAC ATG ACG-3′
2	GCA GTA CG**X** TAC ATG ACG
3	GCA GTA CGT **X**AC ATG ACG
4	GCA G**X**A CGT TAC ATG ACG
5	GCA GTA CGT TAC A**X**G ACG

ODN	sequence
1′	3′-CGT CAT GCA ATG TAC TGC-5′
2′	CGT CAT G**Y**A ATG TAC TGC
3′	CGT CAT G**Z**A ATG TAC TGC

Migration of the excess electron appears to remain within the DNA duplex because cleavage was not significantly inhibited by trapping agents. Irradiation of ODN 3/ODN 2′ for 5 min typically generated the T_9 fragment in 20% yield. An equivalent yield (21%) was similarly generated in the added presence of nitrous oxide (sat.) and mannitol (0.2 mM) used to scavenge hydrated electrons and hydroxyl radicals, respectively (Figure 1b).[18] The results of excess

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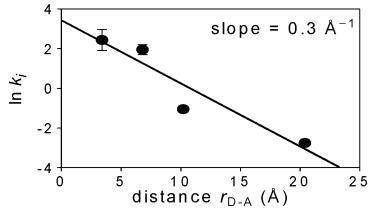

Figure 2. Distance dependence of electron transfer from the excited state of the electron donor to 5-bromo-2′-deoxyuridine. Initial rates of strand cleavage at T_9 (k_i) were obtained from single-exponential curves for duplexes ODN 2′ + ODN 2−5 (0.1 μM) (Supporting Information).

electron transfer were also observed under aerobic conditions, and cleavage at T_9 decreased only marginally to a yield of 16% after 5 min of irradiation (Supporting Information). To our knowledge, this represents the first DNA adapted for long-range transfer of excess electrons that functions in the presence of O_2. Accordingly, this unique donor−acceptor system may be useful for investigating excess electron transfer under physiological conditions, and the donor may additionally find application in nanodevices.

The naphthalene-based donor most likely binds within the duplex DNA at the abasic site in a manner similar to that already shown for an acridine conjugate.[19] The melting temperature (T_m) of the donor containing duplex ODN 1/ODN 2′ ($T_m \approx 57$ °C) is somewhat lower than that of the native duplex ODN 1/ODN 1′ ($T_m = 63$ °C), but higher than that of the duplex lacking the donor ODN 1/ODN 3′ ($T_m = 50$ °C) (Supporting Information). This type of thermal stabilization is consistent with intercalation of the chromophore. Additionally, localized binding at the abasic site can be expected because even the free donor TMDN induced selective reaction at T_9 of a duplex containing BrdU (ODN 3) and an abasic site in place of the donor conjugate (ODN 3′). In contrast, TMDN induced only nonspecific reaction with the fully complementary duplex ODN 3/ODN 1′ (Supporting Information).

In general, electron transfer has shown an exponential dependence on distance (r_{D-A}), and Carell and co-workers have obtained perhaps the weakest dependence to date for excess electron transfer under ambient temperature ($\beta_{apparent}$ of 0.1 Å^{-1}).[8] This value is most consistent with a mechanism of thermally activated hopping. However, electron tunneling seems to dominate excess electron transfer at 77 K as observed by Sevilla and co-workers.[4a] Transfer under either condition is certainly more efficient through dA−T base pairs than dG−dC base pairs or dG stacking.[9c,20] The impedance associated with dG−dC pairs may originate from protonation of the radical anion of cytosine by its hydrogen-bonding partner, guanine.[20]

Preliminary effects of distance on excess electron transfer from the TMDN analogue to BrdU were determined by varying the placement of BrdU within a series of otherwise equivalent oligodeoxynucleotide duplexes containing both dG−dC and dA−T base pairs (ODN 2′ annealed alternately to ODN 2 through ODN 5, Chart 1). The intervening 0−5 base pairs represent estimated distances of 3.4−20.4 Å.[21] The yield of cleavage due to BrdU after 5 min of irradiation under ambient conditions decreased from 36% to 1.5% as the distance increased from 3.4 to 10.2 Å (Supporting Information). Plotting the initial rate of strand cleavage (k_i) against the donor−acceptor distance indicates an exponential distance dependence (slope) of 0.3 Å^{-1} for this mixture of base pairs separating the electron donor and acceptor (Figure 2). The weak distance dependence is similarly consistent with a thermally activated hopping mechanism for excess electron transfer. The increased dependence on distance relative to that observed in the system of Carell and co-workers[8] most likely reflects the added presence of intervening dG−dC pairs in our system.[9c,20]

In summary, we have developed a novel DNA assembly containing a TMDN analogue and BrdU that provides an easily accessible electron donor−acceptor system for investigating excess electron transfer in DNA under conditions complementary to those currently under study. Further analysis of the sequence and structure effects on excess electron transfer under aerobic conditions is now in progress.

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Supporting Information Available: Details of organic synthesis, polyacrylamide gel analysis, and melting curves of ODN duplexes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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