Communication

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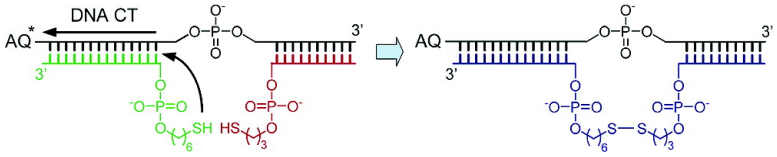

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DNA Charge Transport Leading to Disulfide Bond Formation

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The stacked base pairs within double helical DNA have been shown to mediate charge transport reactions over long distances.[1] Charge transport (CT) through the π-stack has by now been extensively explored both experimentally and theoretically.[2,3] As DNA CT chemistry becomes more clearly understood, another critical issue to examine is the role of DNA CT in cellular mechanisms. DNA CT to yield oxidative damage has been identified within the cell nucleus.[3,4] We have also found that CT occurs in DNA packaged in nucleosomes[5] and that DNA-binding proteins can both inhibit and activate DNA CT.[6] Indeed, DNA-mediated CT may provide a route for long-range signaling among DNA-bound proteins;[7] we have proposed a role for DNA CT in the detection of damage by base excision repair proteins, and guanine radical has been shown to oxidize one repair protein in a DNA-mediated reaction.[7] We have also demonstrated that DNA CT can lead to oxidation of DNA-bound peptides.[8] A possible target of protein oxidation by DNA CT is a cysteine thiol because of its relatively low oxidation potential.[9] Given that the oxidation−reduction cycle of the SH group plays an important role in gene activation and repression,[10] it is essential to elucidate whether DNA CT may also mediate thiol redox chemistry. Here, we show that DNA CT can lead to the oxidation of thiols to form disulfide bonds.

We designed a DNA assembly containing anthraquinone (AQ) as photooxidant spatially separated on the duplex from two SH groups incorporated in the backbone to examine whether DNA CT mediates disulfide bond formation (Figure 1). DNA CT is anticipated to result in the remote oxidation of the SH groups, which are easier to oxidize than the DNA bases.[11]

DNA modified with AQ containing a C_2 alkyl linker (AQ-DNA) was synthesized by a modified procedure.[12] SH groups were attached at the 5′ and 3′-ends of two contiguous DNA strands by using 5′-thiol and 3′-thiol modifiers (5′-SH-DNA and 3′-SH-DNA in Figure 1).[13] Solutions of duplex DNA were prepared by mixing equimolar amounts of each strand plus a complementary 24-mer strand, and then solutions were degassed by freeze−pump−thaw (3×). AQ-modified DNA assemblies were irradiated at 350 nm. After photolysis, methylmethanethiosulfonate (MMTS) was added to cap any unreacted thiol.[14,15]

Figure 2 shows the reverse phase HPLC profiles before and after irradiation. Three peaks (retention time = 10.4, 12.2, and 14.5 min) are assigned to 3′-S-SMe-DNA, AQ-DNA, and 5′-S-SMe-DNA, respectively. Of interest, a new peak appears upon irradiation (retention time = 11.8 min), concomitant with the decrease of the two SH-DNA peaks. From MALDI-TOF MS analysis, this new peak is assigned to the longer DNA (SS-DNA) in which the two shorter DNA pieces (5′-SH-DNA and 3′-SH-DNA) are linked by a disulfide bond ([M − H]$^-$ calcd for SS-DNA, 7612.8; found, 7613.0). The formation of SS-DNA and the disappearance of SH-DNA's as a function of irradiation are also shown. With irradiation, SS-DNA increases while SH-DNA decreases; without AQ irradiation at 350 nm, no reaction is observed. The ratio of SS-DNA formed versus 5′-SH-DNA reacted for DNA1 after 1 min is ~80%.

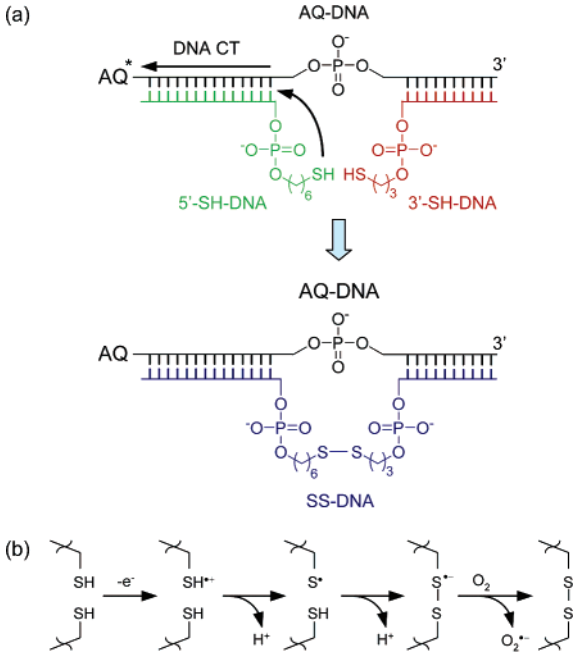

Figure 1. Schematic representation of disulfide bond formation through DNA-mediated charge transport with assemblies used (a) and a plausible reaction scheme (b).

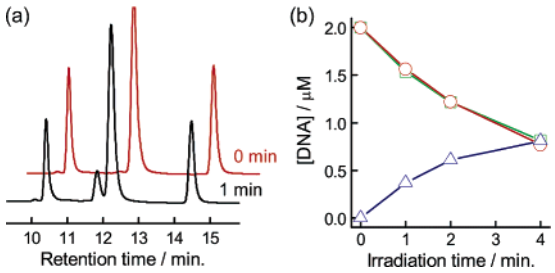

Figure 2. HPLC analysis of disulfide formation. (a) Reverse phase HPLC profiles monitored at 280 nm for DNA1 before (red) and after 1 min irradiation at 350 nm (black). Conditions as in Figure 1. Irradiated sample contains 2 μM DNA, 100 mM NaCl, 20 mM Na phosphate, pH 7.0. (b) Plots of the concentration of 5′-SH-modified DNA (green squares), 3′-SH-modified DNA (red circles), and product disulfide DNA (blue triangles) as a function of irradiation.

These data are consistent with oxidation of the SH group through DNA CT to promote its selective reaction with the neighboring SH group to form the disulfide bond.[16] A plausible mechanism involves SH radical cation generation by DNA CT, release of a proton due to its high acidity, and reaction with the nearby SH to give a disulfide radical anion, accompanied by proton release;[17] the disulfide bond may form with removal of an electron by adventitious oxygen (Figure 1).[18]

Reaction efficiencies for different sequences after 1 min irradiation are given in Table 1. The efficiency for DNA2, where two

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Table 1. DNA Sequences and Yields of Disulfide Formed

duplex		sequence[a]	yield[b] (%)
DNA1	AQ-	5′-AAAT-CAGCACTGTG-GTCGATGCAT-3′ 3′-TTTA-GTCGTGACA***C C***AGCTACGTA-5′	17 ± 3
DNA2	AQ-	5′-AAAT-CAGCACCCTA-ATCCCTGCAT-3′ 3′-TTTA-GTCGTGGGA***T T***AGGGACGTA-5′	24 ± 2
DNA3	AQ-	5′-AAAT-**C**AGCACTGTG-GTCGATGCAT-3′ 3′-TTTA-**A**TCGTGACA***C C***AGCTACGTA-5′	9.5 ± 0.5
DNA4	AQ-	5′-AAAT-CAGC**A**CTGTG-GTCGATGCAT-3′ 3′-TTTA-GTCG**C**GACA***C C***AGCTACGTA-5′	31 ± 4
DNA5	AQ-	5′-AAAT-**C**AGCACCCTA-ATCCCTGCAT-3′ 3′-TTTA-**A**TCGTGGGA***T T***AGGGACGTA-5′	5 ± 1
DNA6	AQ-	5′-AAAT-CAGC**A**CCCTA-ATCCCTGCAT-3′ 3′-TTTA-GTCG**C**GGGA***T T***AGGGACGTA-5′	32 ± 2

[a] Conditions used are as in Figure 2. [b] Yields represent the reaction efficiency obtained from HPLC peak area of the product disulfide DNA after 1 min irradiation.

thiol groups are placed between two GGG sites, is higher than that of DNA1. It is likely that the SH oxidation proceeds through direct collision between SH groups and the DNA bases. The increased reaction efficiency for DNA2 may, in part, be the result of the GGG site serving as a hole sink, facilitating hole transport to the SH groups.[19]

SS-DNA is formed through a DNA-mediated process rather than through an intermolecular reaction involving, for example, superoxide radical anion. That the reaction is intraduplex was determined by examining interduplex controls.[20] The conversion efficiency after 1 min irradiation for these interduplex controls is <2%. Thus, the disulfide bond is produced through an intraduplex process; the reaction occurs from a distance and is DNA-mediated.

We also probed whether the reaction is mediated by the base pair stack by examining the effects of intervening base mismatches. When a CA mismatch is incorporated at a site intervening AQ and the thiols (DNA3 and DNA5), the reaction efficiency is considerably decreased compared to the fully matched duplex; this result is consistent with earlier findings that DNA mismatches perturb DNA CT efficiency.[21] Significantly, we find that introduction of a mismatch at an alternate intervening position closer to the thiols (DNA4 and DNA6) *increases* the reaction efficiency. These results may be explained by the influence of the mismatch on local DNA dynamics; mismatched base pairs may cause a disruption of local structure with an enhancement of conformational motion and fraying.[22] Given that some dynamic motion of the thiol is required to accept a hole from DNA to react further, it is reasonable that enhanced motion associated with the mismatch near the thiols might enhance the reaction efficiency.[23] In any case, any effect of the intervening mismatch, either positive or negative, is an indication that the reaction is DNA-mediated. It is interesting that here, where the reaction is on the backbone rather than within the base stack, stacking perturbations do not necessarily lead to an inhibitory effect.[24]

Therefore, we have shown that DNA CT can lead to reaction on the DNA backbone to produce disulfide bond formation. Given that disulfide bond formation serves as a redox switch in the activation/deactivation of many transcriptional regulators, redox chemistry from a distance mediated by the DNA base pair stack to form disulfides might also now be considered.

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Supporting Information Available: HPLC profiles for DNA1 after irradiation in aerobic and anaerobic conditions, MALDI-TOF spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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