Electron Transfer in DNA

Criteria for Efficient Transport of Excess Electrons in DNA

*Takeo Ito and Steven E. Rokita**

Extensive studies on charge transfer in DNA[1–6] have provided significant insight into the molecular mechanisms of DNA damage induced by high- and low-energy radiation[7] and by chemical oxidants.[8] Appreciation of the conducting properties of DNA has also inspired the development of new nanoscale intelligent materials and chemical sensors.[9] Results from both theoretical and experimental approaches now seem to suggest that electron-deficient intermediates generated from one-electron oxidation of DNA can undergo hole transfer (HT) between guanine (G) residues by single-step tunneling (G hopping), by multistep tunneling through intervening adenine (A) residues (A hopping), and by polaron-like hopping of delocalized radical cations.[1] Much less is known about the complementary process of excess electron transfer (EET) in DNA.[2–6] The results of early experiments involving pulse radiolysis, EPR, and free nucleotides suggested that excess electrons can associate with duplex DNA and migrate between its bases.[10] More recently, Sevilla and co-workers observed that EET proceeds through single-step tunneling at 77 K, but at higher temperatures (>150 K) thermally activated multistep hopping predominates.[3] Cytosine (C) and thymine (T) are most likely to serve as the primary carriers for EET if reduction potentials alone are considered. However, protonation, and consequent stabilization, of the radical anion of C ($C^{-\bullet}$) by water or a Watson–Crick base-paired G residue to form the neutral radical ($CH^{\bullet}$) may inhibit migration.[2a,3c,10–13]

Only limited information on the effects of context on EET has so far been reported and many basic questions remain to be addressed before a detailed understanding of the structural dependence of this process can be established at a level comparable to that previously reached for HT. Carell and co-workers have driven the repair of thymine dimers by EET from a reduced flavin alternately coupled by inter- and intrastrand attachment.[5] In each case, efficient EET through A/T base pairs was apparent and the dependence of the transfer on distance was weak. Initial data on the dynamics of EET and charge recombination have also begun to emerge from a series of stilbene-capped hairpin DNA molecules and pyrenyl–oligodeoxynucleotide (ODN) conjugates.[4]

Our research group has developed a complementary system based on ODN conjugates containing a derivative of

[*] Dr. T. Ito, Prof. S. E. Rokita
Department of Chemistry and Biochemistry
University of Maryland
College Park, MD 20742 (USA)
Fax (+1) 301-405-9376
E-mail: rokita@umd.edu

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N,*N*,*N*′,*N*′-tetramethyl-1,5-diaminonaphthalene (TMDN) and 5-bromo-2′-deoxyuridine (^{Br}U; Figure 1).[6] Selective photoexcitation of the donor TMDN (> 335 nm, E^*_{ox} = ca. −2.8 V versus a saturated calomel electrode) initiates EET, and the

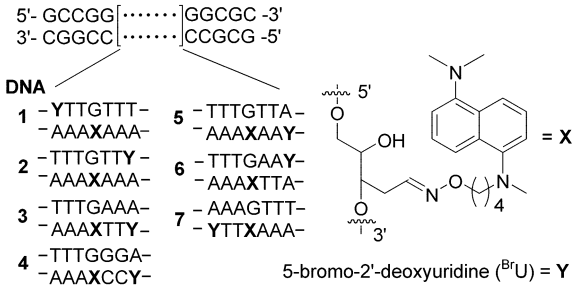

Figure 1. Oligodeoxynucleotide sequences and sensitizer structure.

subsequent reduction of the acceptor ^{Br}U promotes decomposition of its 5′ neighbor. This decomposition in turn leads to an easily detectable strand fragmentation after treatment with hot piperidine (Figures 2 and 3).[14] The extent of fragmentation depends on the duration of UV exposure, as was

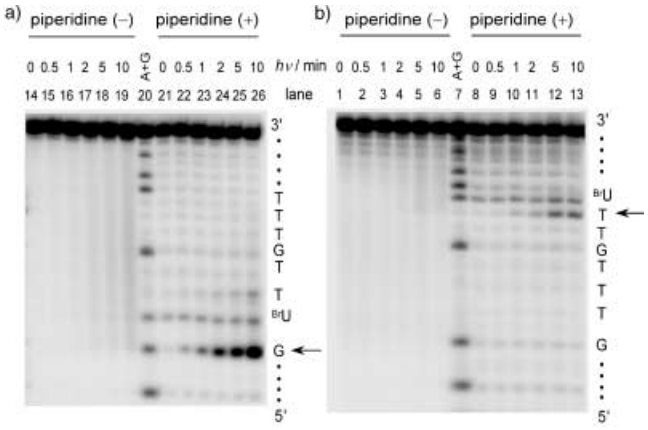

Figure 3. Autoradiograms of 20% denaturing polyacrylamide gels showing strand scission of DNA after UV irradiation (> 335 nm, around 10 °C). Two 5′-^{32}P-labeled ODNs containing ^{Br}U were examined: a) DNA **1** and b) DNA **2** (1 mM, 90 nCi) in sodium phosphate (10 mM, pH 7.0) and NaCl (90 mM). The ODNs were exposed to UV light for the indicated periods and analyzed either directly (lanes 1–6 and 14–19) or after subsequent treatment with 10% piperidine at 90 °C for 30 min (lanes 8–13 and 21–26). The arrows highlight products formed by EET.

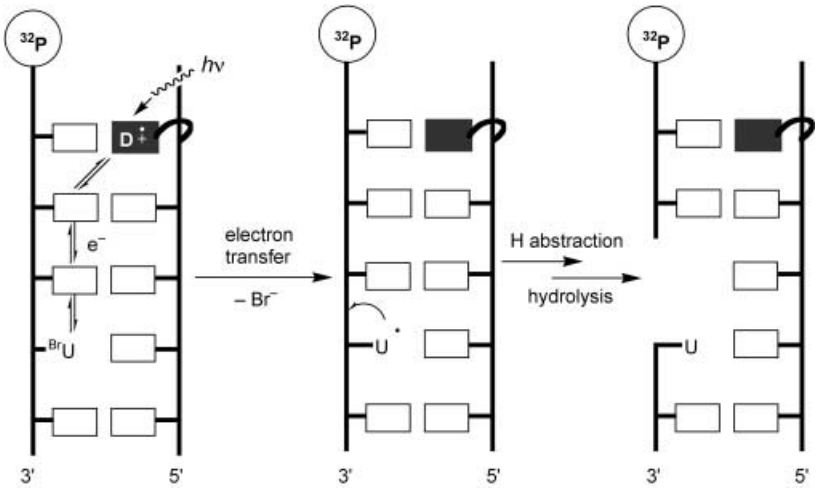

Figure 2. EET from an *N*,*N*,*N*′,*N*′-tetramethyl-1,5-diaminonaphthalene analogue (D) to 5-bromo-2′-deoxyuridine (^{Br}U) in DNA.

expected; irradiation alone does not induce comparable fragmentation (Figure 3). Weak cleavage at ^{Br}U is also evident after piperidine treatment without irradiation but this background reaction is independent of UV exposure. As illustrated in Figure 3, the apparent efficiency of EET can differ significantly between closely related duplexes (**1** and **2**). A series of similar duplexes containing donor and acceptor residues separated by a constant distance was examined for sequence dependence and sensitivity of EET to 1) proton transfer, 2) intra- and interstrand hopping, and 3) 3′ to 5′ or 5′ to 3′ directionality.

The initial rates of strand fragmentation generated by irradiation at wavelengths above 335 nm were used to compare the relative efficiency of EET from the attached TMDN analogue to the ^{Br}U residue in various DNA molecules (Figure 4). Substitution of two intervening A/T base pairs (**3**) with G/C pairs (**4**) suppressed EET in H_2O more than fourfold. This result is consistent with the hypothesis that T is the primary carrier of charge.[2a] As mentioned above, any contribution by C might be limited by preferential protonation of $C^{-\bullet}$ rather than $T^{-\bullet}$. If proton transfer is indeed competitive with EET, then the rate of strand fragmentation as a result of EET should be enhanced through G/C, but not through A/T base pairs when the solvent is changed from H_2O to D_2O.[3c] This enhancement was confirmed by the observation of an inverse solvent isotope effect for the G/C-containing DNA **4** ($k_{H_2O}/k_{D_2O} = 0.68$) and neglible such effects for the A/T- and T/A-containing DNAs **3** and **5** ($k_{H_2O}/k_{D_2O} = 0.97$ and 1.1, respectively; see Figure 4).

Additional parameters that affect EET were identified simply by rearranging the ^{Br}U residue and the two intervening A/T base pairs within an otherwise common duplex structure (Figure 1). For example, by switching the A/^{Br}U base pair of **3** to give ^{Br}U/A (**6**), or alternatively, switching AAA/TT^{Br}U in **3** to TT^{Br}U/AAA (**2**), we enforced a single crossover and interstrand capture of an electron. In both examples, EET was greatly inhibited in the interstrand systems compared to the intrastrand ones (Figure 4). However, generalization of the effect of interstrand electron capture on EET is made difficult by the implicit change in strand directionality involved, as illustrated by DNAs **2** and **3**. Little change in the efficiency of EET was observed when 3′ to 5′ directionality was preserved (compare **1** and **3**), which shows that a single crossover between strands may modulate, but does not dominate, EET. Previous experimental analysis of HT in a series of defined DNA sequences revealed a preference for intrastrand arrangements

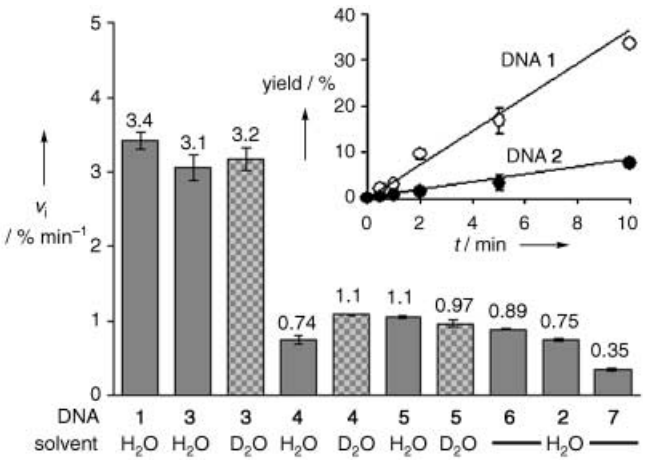

Figure 4. EET in DNAs **1**–**7** as indicated by strand scission after UV irradiation and piperidine treatment. Initial fragmantation rates (v_i) were obtained by fitting cleavage curves (% scission product plotted against total material) to first-order exponential curves (see the Supporting Information). Each analysis was repeated at least three times. Inset: Cleavage of DNA **1** (open circles) and DNA **2** (closed circles) as a function of irradiation time.

of the donor, acceptor, and bridging residues, although multiple crossovers were still possible.[15]

HT has been noted to be dependent on strand directionality[15b] and EET also appears to be highly sensitive to this aspect of the structure, as highlighted by a comparison of DNAs **3** and **7**. These two DNAs are nearly identical: both contain the same donor and acceptor and two bridging T residues, all within a single strand (Figure 1). However, a change in the orientation of the acceptor and bridge from 5′ to 3′ with respect to the donor caused a dramatic decrease of almost ninefold in EET efficiency. The bias in favor of EET in a 3′ to 5′ direction was evident in DNA containing the donor and acceptor on complementary strands (**1** and **2**) as well, although the equivalent decrease in efficiency was around 4.5-fold (Figures 3 and 4). HT favors migration in the opposite direction (5′ to 3′),[15b] but this process also entails electron migration in the 3′ to 5′ direction. An asymmetry in the HOMO overlap of nucleobases acting as charge carriers has been proposed as an explanation for the directionality of HT[15b] and an equivalent proposal based on LUMO overlap could be considered for EET. Variations in the electron affinity of ^{Br}U caused by changes in its flanking nucleotides may also contribute to the observed transfer efficiencies but, at least for DNAs **3** and **7**, the difference between transfer through 5′-C^{Br}UT and that with 5′-T^{Br}UC is predicted to be insignificant.[16]

In summary, the photochemical reactivity of DNAs **1**–**7** helped to delineate the major influence of DNA sequence on EET. We suspect that protonation of $C^{-\bullet}$ limits participation of C and leaves T as the only major conduit. Strand crossing and, particularly, orientation can also significantly influence the efficiency of EET. Such information could be used to refine designs of molecular wires that utilize DNA self-assembly.

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