# Long-Distance Charge Transport in DNA: Sequence-Dependent Radical Cation Injection Efficiency

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Interest in the oxidation of DNA stems in part from the observation that the removal of an electron (formation of a radical cation) damages bases that are far away from the site of charge injection.[1−9] This fact has led to speculation that DNA may be a one-dimensional conductor[10,11] and that this feature plays a role in metabolic processes associated with mutations and their repair.[12,13] Embedded in these studies are complexities associated with base sequence effects. For example, oxidative reactions typically result in damage to 5′-guanines that are contained in remote GG or GGG sequences.[1,6,14−16] Giese and co-workers have shown that sequences of three or four contiguous A or T bases inhibit remote reaction when they are interposed between the site of charge injection and a GGG target.[3,17] On that basis, they proposed a mechanism for radical cation transport that proceeds through oxidation of intervening G bases.[5,18] In contrast, we found that charge transport through contiguous (A/T) sequences is not significantly less efficient than transport through comparable sequences containing all four bases.[6−8] These findings led to our postulation of the phonon-assisted polaron hopping model, which is finding support.[19] We report herein experiments that reveal the effect of base sequence on the efficiency of radical cation injection. The results show that sequence effects depend strongly on the nature of the competing reactions.

We have established that photoinduced oxidation results from irradiation of an anthraquinone derivative (AQ) that is linked covalently to a 5′-terminus of duplex DNA oligomers. The AQ is end-capped, and its excited state accepts an electron from the DNA in an exothermic reaction that generates the anthraquinone radical anion and a base radical cation. Migration of the radical cation through the DNA is revealed as strand cleavage, primarily at the 5′-G of GG sequences, following treatment with piperidine.[9]

**Table 1.** Reactivity Data for AQ-DNA

| AQ-DNA | reaction time[a] | ($GG_p$/$GG_d$)[b] | $\Phi(_{-DNA})$[c] | AQ-DNA | reaction time[a] |
|---|---|---|---|---|---|
| **1** | 5 | 14 | 4.5 | **5** | 5 |
| **2** | 5 | | | **6** | 8 |
| **3** | 500 | | | **7** | 80 |
| **4** | 500 | 6 | 0.03 | **8** | 50 |

[a] Irradiation time in minutes required for approximately equivalent reaction at $GG_p$ as determined by PAGE. [b] Ratio of strand cleavage at $GG_p$ and $GG_d$ recorded at 1 and 120 min of irradiation, respectively. At the long irradiation times, we suspect that minor nondistance-dependent processes (for example, $^1O_2$) complicate the data. [c] Quantum yield (%) for disappearance of DNA after irradiation at 350 nm and treatment with piperidine. The estimated error from independent replication is ±10% of the reported value.

The compounds we investigated (see Figure 1) were designed to test the effect of base sequence on the efficiency of charge injection by the AQ.

The compounds studied contain a variable four base pair sequence ($N_1$-$N_4$/$X_1$-$X_4$) adjacent to the covalently linked AQ, and two GG steps ($GG_p$ and $GG_d$), which serve as indicators of radical cation injection. These oligomers were prepared by automated solid-phase synthesis, and the complementary strand (non-AQ containing) was 5′-labeled with $^{32}P$. The samples were irradiated (350 nm, only the AQ absorbs) at room temperature in a phosphate buffer solution (pH = 7.0), treated with hot piperidine, and then analyzed by polyacrylamide gel electrophoresis.[20] The gels were visualized by autoradiography, and the cleavage efficiency was quantified by phosphorimagery. Gels from the investigation of DNA(1) and DNA(4) are shown in Figure 2, all data are summarized in Table 1.

The effect of base sequence on reaction efficiency is remarkable. Strand cleavage is readily detected at $GG_p$ and $GG_d$ for DNA(1) after 5 min of irradiation; but, 120 min are required to yield measurable reaction of DNA(4). The distance dependence for charge migration, determined from the ratio of cleavage at $GG_p$ to $GG_d$, is consistent with previously reported values (∼0.02 $Å^{-1}$).[1,7,8] Clearly, the efficiency of charge injection by the AQ is extraordinarily sensitive to the sequence of bases in positions near the AQ.

Comparisons of DNA(1) with DNA(5) and DNA(3) with DNA(4) show that the efficiency of reaction is not dependent upon whether the AQ is attached to the terminal purine, as it is in DNA(1,3), or the terminal pyrimidine, as in DNA(4,5). This is consistent with models of the end-capped AQ, which show overlap with both terminal bases.[6] It is reasonable to presume that it is the terminal purines that are oxidized to give the radical cations, since their $E_{ox}$ are lower than those of the pyrimidines.[21] These findings indicate that a G/C base pair at the terminal position is a feature that reduces the charge injection efficiency.

DNA(6) is identical to DNA(4) except that the terminal G on the complementary strand is deleted. The efficiency of reaction for DNA(6) is nearly the same as that of DNA(1) and DNA(2). This result plainly shows that a guanine in the terminal position adjacent to the AQ results in low charge injection efficiency. The effect of guanine position was probed further by placing it at either the second or third positions in DNA(7) and DNA(8), respectively. The data reveal that increasing the distance between the first G and the AQ increases the efficiency of radical cation injection. These trends were quantified by measurement of the quantum yield for consumption ($\Phi_{-DNA}$) of DNA(1) and for DNA(4) using

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| AQ-5'- | $N_1$ | $N_2$ | $N_3$ | $N_4$ | G | C | C | G | G | T | A | C | A | A | A | C | A | T | G | G | C | C | G | T | A | C | G | -3' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3'- | $X_1$ | $X_2$ | $X_3$ | $X_4$ | C | $G_p$ | $G_p$ | C | C | A | T | G | T | T | T | G | T | A | C | C | $G_d$ | $G_d$ | C | A | T | G | C | -5' |

| AQ-DNA | $N_1$-$N_4$/$X_1$-$X_4$ | AQ-DNA | $N_1$-$N_4$/$X_1$-$X_4$ |
|---|---|---|---|
| 1 | AAAT/TTTA | 5 | TAAT/ATTA |
| 2 | TTTA/AAAT | 6 | CAAT/—TTA |
| 3 | GAAT/CTTA | 7 | ACAT/TGTA |
| 4 | CAAT/GTTA | 8 | AACT/TTGA |

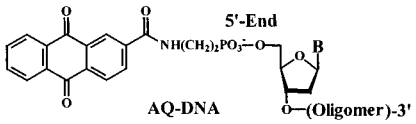



**Figure 1.** Structures of DNA oligomers.

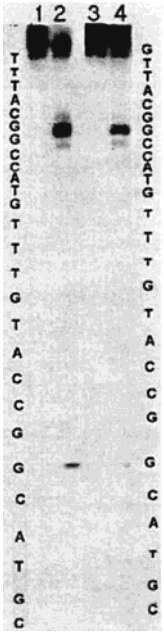



**Figure 2.** Autoradiogram from irradiation of DNA(1) for 5 min, lane 2, and DNA(4) for 500 min, lane 4. Lanes 1 and 3 are controls. All samples were treated with piperidine.

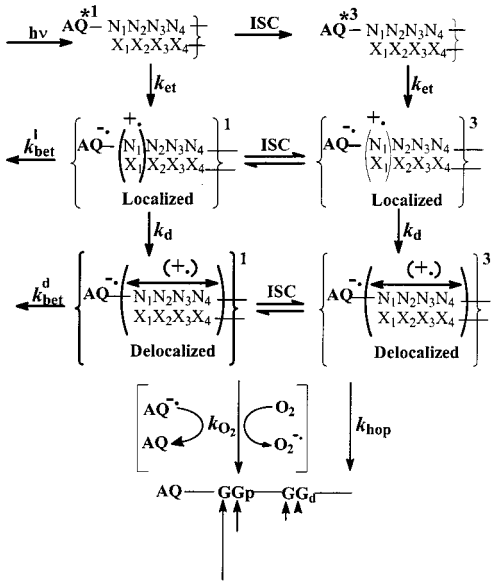
anthraquinone sulfonate as an actinometer.[22] Cleavage of DNA-(1) is ~150 times more efficient than reaction of DNA(4).[20]

The efficiency of reaction at the remote GG steps in these DNA oligomers is strongly dependent on the sequence of base pairs near the AQ. The reaction mechanism outlined in Scheme 1 accommodates this finding. Irradiation leads to the excited singlet state ($AQ^{*1}$), which may oxidize the DNA ($k_{et}$) or intersystem cross (ISC) to the triplet excited state. Oxidation by $AQ^{*1}$ gives radical ion pairs in an overall singlet state, while $AQ^{*3}$ gives triplet radical ion pairs. We propose that the reactions of these radical ion pairs control the sequence dependent efficiency.

Immediately after it is formed, the radical cation is localized on the terminal purine ($N_1$ or $X_1$). According to the phonon-assisted polaron hopping model, the DNA responds rapidly ($k_d$) to charge injection by forming a distorted structure (the polaron) that stabilizes the charge and delocalizes it over several base pairs. Back electron transfers ($k^l_{bet}$ and $k^d_{bet}$) result in consumption of the radical cation with no net reaction of the DNA. If the ion pairs avoids back electron transfer, the $AQ^{-\bullet}$ is consumed by $O_2$ ($k_{O_2}$), which results in formation of superoxide ($O_2^{-\bullet}$)[23] and allows the polaron to hop ($k_{hop}$) to remote sites where it reacts.

It is unlikely that the rate of back electron transfer for the localized radical cation ($k^l_{bet}$) will vary significantly with the identity of the terminal purine since, according to Marcus theory, this value depends on the reorganization energy ($\lambda$) and $\Delta G_{et}$, which differ only modestly for A and G.[24] However, back electron transfer will be much slower when the radical cation is delocalized ($k^d_{bet}$), since its magnitude is strongly distance-dependent.[25]

**Scheme 1.** Sequence Effect on Charge Injection




Delocalization ($k_d$) requires a structural distortion of the DNA. When the terminal base pair contains a G (lowest $E_{ox}$), as it does in DNA(3,4), delocalization of the charge will be slower and $k^l_{bet}$ will compete more effectively with $k_d$ resulting in lower cleavage efficiency. On the other hand, $k^d_{bet}$ should vary in the order DNA(4) > DNA(7) > DNA(8), and thus $k_{O_2}$ will become more competitive and the reaction efficiency will increase in the reverse order, which is observed. These conclusions are summarized in eq 1, where $R$ is a proportionality constant.

$$\Phi_{(-DNA)} = [R][k_d/(k_d + k^l_{bet})\cdot k_{O_2}/(k_{O_2} + k^d_{bet})] \qquad (1)$$

These findings illustrate that the efficiency of charge injection in DNA depends strongly and sensibly on the sequence of base pairs. They are particularly relevant to Giese's observation[3,17] of a base sequence effect different from that reported here. However, back electron transfer is not possible in their method of radical cation generation, and thus similar sequence effects are not to be expected. These results also highlight the necessity for examination of well-defined DNA structures. The powerful effect of base sequence on the charge injection can result in widely varying reaction efficiencies for adjacent intercalation sites. Thus, experiments with oxidants that are not covalently bound,[26] and those with bound oxidants on long tethers capable of accessing several binding sites[27,28] may be difficult to interpret.

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**Supporting Information Available:** Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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