## Variations in DNA Charge Transport with Nucleotide Composition and Sequence

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Long-range oxidative damage to DNA has been demonstrated in experiments using a variety of remotely bound oxidants.[1−5] However, the mechanism(s) by which charge is transported through the base pair stack needs still to be established. Recent theoretical proposals bring together tunneling and hopping mechanisms to describe charge transport.[6] On the basis of measurements of damage yield, it has been proposed that charge transport occurs by hopping between guanine sites and tunneling through TA steps.[7] In accord with guanine hopping, oxidative damage over long distances was not observed when 5′-TATATA-3′ intervened between G sites.[8] Phonon-assisted polaron hopping has been suggested as an alternative mechanism.[9] In this model, the sequence-dependent conformational dynamics of DNA are expected to aid in charge transport.

These different proposals have led us to investigate systematically the effect of intervening base composition and sequence on long-range oxidative DNA damage. Here, we vary the intervening sequence between two oxidatively sensitive sites without varying overall base composition. Oxidative damage can occur up to 200 Å from the site of hole injection; sequence-dependent effects were attributed to variations in sequence-dependent structure and flexibility.[10] Recent ultrafast spectroscopic studies have shown that base dynamics may gate charge transport,[11] and fluorescence studies on DNA assemblies containing bound donors and acceptors have underscored the sensitivity of fluorescence quenching to stacking.[12]

Table 1 shows substrates designed to examine long-range charge transport through sequences rich in AT base pairs. Each sequence contains two 5′-**G**G-3′ doublets,[13] one proximal and one distal to the tethered intercalating photooxidant, Rh(phi)$_2$bpy′ $^{3+}$ (phi = phenanthrenequinone diimine);[15] the rhodium complex promotes damage to the 5′-G of the guanine doublet by photoinduced electron transfer. Irreversible trapping of the guanine radical by $H_2O$ and $O_2$, once generated, is assumed to be independent of variations in the global DNA sequence, since each 5′-C**G**GC-3′ site is identical in its local sequence context. The ratio in yield of damage at the 5′-G of the 5′-GG-3′ for the distal versus proximal sites then provides a measure of relative transport efficiency through the intervening sequence.[16] The damage yield is determined by treatment of the 5′-$^{32}$P-labeled oligonucleotide with piperidine, followed by polyacrylamide gel electrophoresis and phosphorimagery.[1,17−19]

**Table 1.** Long-Range Oxidative Damage in DNA Sequences Functionalized with the Tethered Photooxidant Rh(phi)$_2$bpy′ $^{3+}$

| Sequence [a] | Assembly | Distal/Proximal Oxidation Ratio [b] |
|---|---|---|
| AC Rh GAGCCGAAAAGCCGTAT-3′<br>3′-TG CTCGGCTTTTCGGCATA-5′* | TT-2 | 0.9 ± 0.1 |
| AC Rh GAGCCGTTTTGCCGTAT-3′<br>3′-TG CTCGGCAAAACGGCATA-5′* | AA-2 | 2.5 ± 0.2 |
| AC Rh GAGCCGTATAGCCGTAT-3′<br>3′-TG CTCGGCATATCGGCATA-5′* | AT-2 | 0.6 ± 0.2 |
| AC Rh GAGCCGAAAAAGCCGTAT-3′<br>3′-TG CTCGGCTTTTTTCGGCATA-5′* | TT-3 | 1.2 ± 0.3 |
| AC Rh GAGCCGTTTTTGCCGTAT-3′<br>3′-TG CTCGGCAAAAAACGGCATA-5′* | AA-3 | 3.5 ± 0.5 |
| AC Rh GAGCCGTATATAGCCGTAT-3′<br>3′-TG CTCGGCATATATCGGCATA-5′* | AT-3 | 1.0 ± 0.2 |
| AC Rh GAGCCGAAAAAAGCCGTAT-3′<br>3′-TG CTCGGCTTTTTTTCGGCATA-5′* | TT- 4 | 2.2 ± 0.4 |
| AC Rh GAGCCGTTTTTTTGCCGTAT-3′<br>3′-TG CTCGGCAAAAAAACGGCATA-5′* | AA-4 | 2.3 ± 0.1 |
| AC Rh GAGCCGTATATATAGCCGTAT-3′<br>3′-TG CTCGGCATATATATCGGCATA-5′* | AT-4 | 1.8 ± 0.2 |
| AC Rh GAGCCGAAAAAAAAAGCCGTAT-3′<br>3′-TG CTCGGCTTTTTTTTTTCGGCATA-5′* | TT-5 | 0.4 ± 0.3 |
| AC Rh GAGCCGTTTTTTTTTTGCCGTAT-3′<br>3′-TG CTCGGCAAAAAAAAAACGGCATA-5′* | AA-5 | 1.2 ± 0.1 |
| AC Rh GAGCCGTATATATATAGCCGTAT-3′<br>3′-TG CTCGGCATATATATATCGGCATA-5′* | AT-5 | 1.3 ± 0.1 |
| AC Rh GAGCCGTATAGCTATAGCCGTAT-3′<br>3′-TG CTCGGCATATCGATATCGGCATA-5′* | TAGC | 0.6 ± 0.1 |

[a] Sequences isolated and purified as described previously.[13] Only the Δ diastereomer was used. The photooxidant is shown intercalated into its primary binding site based on the photocleavage patterns. * denotes the site of $^{32}$P labeling. [b] Conditions are described in Figure 1. Values represent averages of three trials.

Figure 1 shows the phosphorimagery after photooxidation of AA-2, TT-2, and AT-2. For these assemblies, the base pair composition between proximal and distal guanine doublets is constant, although the sequence of bases varies. If the mechanism of charge transport were strictly a function of hopping between guanine sites,[6,7] one would expect the distal/proximal ratio of oxidative damage for these assemblies to be equal. On the basis of data obtained by others for 5′-ATAT-3′,[7] little distal oxidation might be expected for all assemblies.[20] As is evident in Figure 1

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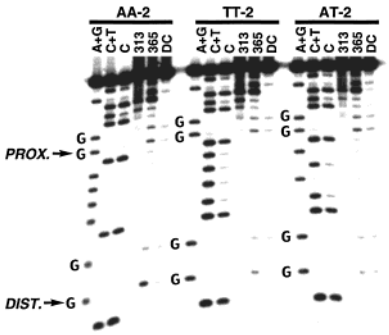


**Figure 1.** Phosphorimagery of a denaturing 20% polyacrylamide gel. Shown are the results from sequences containing four base pairs intervening between distal and proximal guanine doublets, AA-2, AT-2, and TT-2. Sequence designations are as in Table 1. For each assembly, lanes are as follows: A + G, C + T, and C show Maxam−Gilbert sequencing reactions; 313 shows the fragment after direct photocleavage at 313 nm for 10 min without piperidine treatment; 365 nm shows the fragment after irradiation at 365 nm for 1 h at 23 °C, followed by piperidine treatment; DC (dark control) shows samples not irradiated but treated with piperidine. All samples contained 4 $\mu$M metal complex-tethered duplex, 20 mM Tris-HCl, pH 8, 10 mM NaCl. Sites of proximal and distal 5′-GG-3′ damage are indicated.

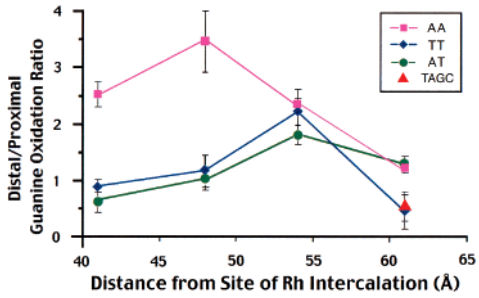


**Figure 2.** Plot of the distal/proximal guanine oxidation ratio versus the distance from the intercalation site, based upon data in Table 1. Distances are estimated from the primary intercalation site, established by direct photocleavage at 313 nm, assuming 3.4 Å stacking.

and quantitated in Table 1, this is not the case. Instead, we find significant distal oxidation and the ratio to be consistently higher for the AA assemblies and lower for the TT and AT assemblies. On the basis of energetic considerations[21] as well as poor stacking overlap, the TT sequences might be expected to be the poorest conduits for charge transport.[22] Similar considerations dictate that adenine tracts should yield efficient charge transport, and duplexes containing AT-tracts might be expected to show damage in the intermediate range.

Table 1 also shows the effect of increasing the length of the intervening segment. On the basis of a guanine hopping model,[6,7] increasing the number of adenines or thymines between the guanine doublets should result in marked decreases in long-range guanine oxidation; indeed, with this mechanism,[7] one would expect negligible oxidative damage at the distal site. However, as is evident in Figure 2, it is the *sequence* of bases that is critical. Increasing the length of the AA sequence only slightly decreases the guanine oxidation ratio, consistent with the shallow distance dependence expected for hole hopping through *all* the bases. Remarkably, in the case of the TT and AT assemblies, there appears to be an *increase* in oxidation ratios with increasing oligonucleotide length from four to eight intervening base pairs.[24,25] Furthermore, in contrast to that predicted by a guanine-hopping model, insertion of a GC step into the otherwise A·T bridge actually *decreases* the efficiency of charge transport (Table 1, TAGC). This result provides clear evidence that strict guanine hopping cannot describe long-range DNA-mediated charge transport in this system.[26] Alternative mechanisms which involve hopping also among other bases are required.[29]

We propose that the variations observed with sequence and length must depend also upon the conformational dynamics associated with these sequences. In contrast to hole hopping models developed primarily for aromatic crystals,[30] here electronic coupling between bases is dynamic and sequence-dependent. For the AA oligonucleotides, the efficiency of charge transport may depend on the extensive overlap of the stacked purines. Moreover, A-tracts are well known to adopt conformations that differ from that of canonical B-form DNA.[31] The increase in damage ratios with increasing length for TT sequences is consistent with the cooperative formation of conformational domains in longer A-tract DNA structures; bends seem to require a nucleating core of five adenines.[32] In our system, convergence of the oxidation ratios occurs in the duplexes containing six or more A·T base pairs between the guanine doublets. Critically, the result we obtain with 5′-TATA-3′, which may contrast previous reports,[7] can now be viewed in a systematic context.[33] As with the A-tracts, the increase in transport efficiency with lengthening of this segment may also reflect some conformational transition associated with the longer, ordered sequence; no precedence for such a finding is available. Rather than considering hopping from guanine to guanine, we might consider *hopping between domains*. Certainly, our results show that a simple guanine hopping model cannot account for charge transport through long sequences of DNA. These observations underscore the impact on DNA charge transport of sequence-dependent conformational domains and their dynamics.

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**Supporting Information Available:** Phosphorimagery of a denaturing 20% polyacrylamide gel showing long-range oxidative damage in DNA assemblies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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