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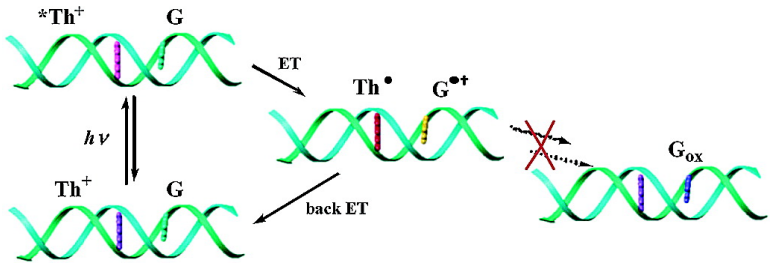
J. Am. Chem. Soc., **2003**, 125 (32), 9586-9587• DOI: 10.1021/ja036397z • Publication Date (Web): 22 July 2003



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Published on Web 07/22/2003

Fast Back Electron Transfer Prevents Guanine Damage by Photoexcited Thionine Bound to DNA

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The genome is continually exposed to oxidative stress, and guanine in particular is the major target because it has the lowest oxidation potential of all bases.[1] Once a radical cation (hole) is generated in DNA, it is able to migrate through the π-stacked base pair array to low energy sites,[2−9] such as 5′-GG-3′ or 5′-GGG-3′ sequences.[10] We have shown that DNA hole transport also occurs in whole nuclei and may thus have biological significance.[11] Although the detailed mechanism remains to be resolved, it appears to involve a mixture of hopping and tunneling elements.[12] Many reagents have been used to generate a hole in DNA, including phenanthrenequinonediimine complexes of rhodium(III),[3] polypyridyl complexes of Ru(II),[4] naphthalimides,[5] anthraquinones,[6] benzophenones,[7] stilbenes,[8] and Norrish reagents.[9] Recently, the phenothiazinium dye thionine has been used for studies of electron transfer with DNA.[13] Upon photoexcitation in the presence of guanine-containing nucleotides or DNA, thionine undergoes a rapid ground state recovery and a dramatic decrease in emission intensity, consistent with reductive quenching by guanine. Here, we provide definitive spectroscopic evidence for electron transfer from guanine to excited thionine but show that there is no permanent damage to guanine from this process. These results can be explained by rapid electron transfer to the guanine radical cation from the reduced form of intercalated thionine and demonstrate that back electron transfer is fundamentally significant to understanding the hole transport chemistry of DNA.

The photooxidation of guanine (**G**) by thionine ($\mathbf{Th}^+$) is depicted in Scheme 1. Excitation produces excited singlet thionine ($^*\mathbf{Th}^+$), which then oxidizes **G** to produce the electron-transfer intermediates, [$\mathbf{Th}^\bullet$, $\mathbf{G}^{\bullet+}$]. These intermediates can react by back electron transfer or undergo cage escape to produce secondary intermediates, $\mathbf{ThH}^{\bullet+}$ and $\mathbf{G}(-\mathbf{H})^\bullet$, that decay on a longer time scale.[14]

We first examined the thionine−guanine photochemistry by sequencing gel electrophoresis of a DNA duplex with two GG sites, where the guanine oxidation would be detected as strand cleavage after piperidine treatment.[1b] Upon irradiation at 599 nm of thionine in the presence of DNA, however, no DNA damage is detected (Figure 1). Similar results are observed for DNA with covalently tethered thionine.[17] Even at high concentrations of thionine and long irradiation times, little damage is observed (Figure 1, lane 4).[18] This absence of damage is remarkable given that the excited thionine singlet has a high enough reduction potential (~2 V)[13,19] to accept an electron from guanine. In fact, it has been reported that quenching of the singlet thionine occurs on the femtosecond time scale in the presence of DNA,[13c] and this quenching is assigned to electron transfer from guanine on the basis of a driving force dependence of the kinetics. However, there has been no direct evidence of electron transfer to form radical intermediates.

Using transient absorption spectroscopy,[20] we characterized the formation of the reduced thionine in the presence of GMP. When thionine is excited by a 599 nm laser pulse in the presence of GMP, we observe a rapid decay and a long-lived transient on the

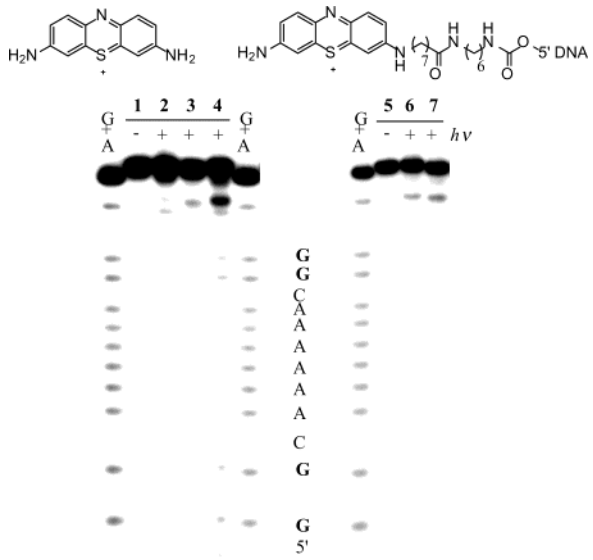
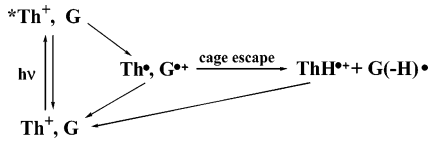


Figure 1. Autoradiograms after denaturing gel electrophoresis of the duplex containing ^{32}P-5′-ATA CGG CAA AAA ACG GCT CGT following photooxidation by free thionine (10 μM in lane 3 and 100 μM in lanes 1, 2, and 4) and covalently bound thionine (lanes 5−7). The duplexes (4 μM) were irradiated at 599 nm in 10 mM Na phosphate, 50 mM NaCl, pH 7.6, for 1 h (lane 6) or 2 h (lanes 2−4 and 7), followed by hot piperidine treatment (90 °C, 20 min). Samples in lanes 1 and 5 are not irradiated, and in lane 2 they are irradiated without piperidine treatment.

Scheme 1



microsecond time scale at 390 nm (Figure 2), where both the neutral guanine radical and the reduced thionine absorb. The size of this signal increases with increasing concentrations of GMP (Figure 2, inset). Monitoring the long-lived signal as a function of wavelength, we obtain the absorbance difference spectrum, with maxima at 390 and 770 nm (Figure 3); a similar spectrum is obtained for thionine in the presence of Fe(II)-EDTA instead of GMP. Both spectra agree closely with the spectrum for reduced, protonated thionine radical.[16] However, for thionine quenched by GMP, the peak at 390 nm contains a shoulder at longer wavelengths and is more intense than that in the reported spectrum.[16] These differences likely reflect the presence of the deprotonated guanine radical, which absorbs light in this region.[15] The existence of these transient species establishes the occurrence of electron transfer.

In marked contrast to the behavior observed with GMP, no transient was observed for thionine bound to poly d(GC). These data show only a spike with a much lower height than that obtained from reactions with or without GMP (Figure 2). These results are

10.1021/ja036397z CCC: $25.00

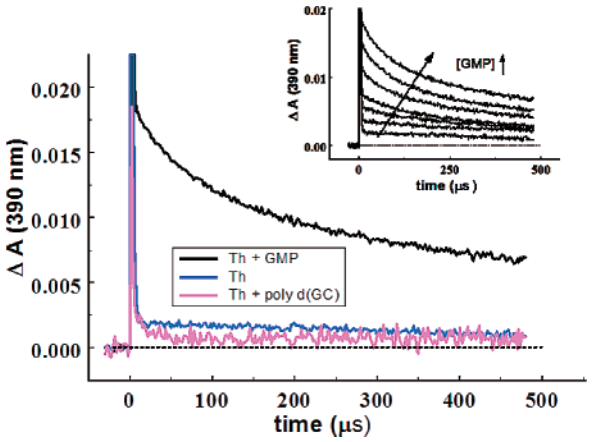


Figure 2. Transients formed upon photoexcitation of thionine. Shown are transient absorption signals at 390 nm for thionine alone (blue), and in the presence of GMP (black) or poly d(GC) (magenta). Inset: growth of the long-lived signal as the concentration of GMP is increased from 0 to 4 mM. Conditions: 25 μM thionine, 4 mM GMP, or 0.5 mM nucleotides poly d(GC), in a buffer of 10 mM Na phosphate, 50 mM NaCl, pH 7.6; excitation was at 599 nm (~7 mJ/pulse) with a YAG-OPO laser.

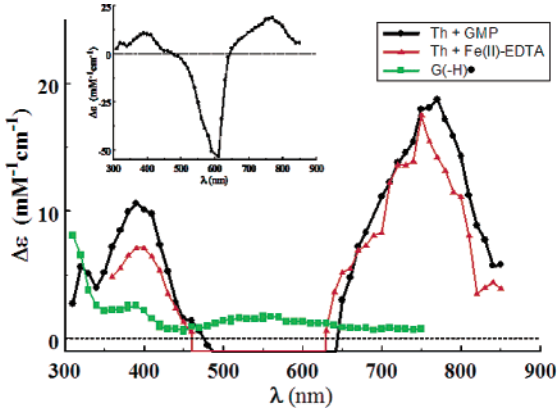


Figure 3. Absorbance difference spectra of long-lived transients formed upon quenching of photoexcited thionine. Shown are spectra for thionine quenched by GMP (circles) or Fe(II)-EDTA (triangles), obtained from the absorbance of the signals at 10 μs; the spectrum of **G**(−**H**)$^{\bullet}$ is from 4a (squares). The negative portion of the spectra is truncated to emphasize the positive bands characteristic of reduced thionine; the inset shows the full spectrum of the thionine/GMP transients, with the large bleach of thionine absorbance near 600 nm. Absorbance values were converted to extinction coefficients using $\epsilon_{390} = 2.6$ mM^{-1} cm^{-1} and $\epsilon_{750} = 0.5$ mM^{-1} cm^{-1} for **G**(−**H**)$^{\bullet}$ [15] and $\epsilon_{750} = 17.5$ mM^{-1} cm^{-1} for **ThH**$^{\bullet+}$.[16] The high absorbance of the metal complex precluded measurements below 360 nm in the reduction of thionine by Fe(II)-EDTA. Conditions: 1 mM GMP or 1 mM Fe(II)-EDTA as quencher; other conditions are as in Figure 2.

consistent with rapid quenching of thionine and indicate that all transient species are short-lived in the presence of poly d(GC), as expected.[13] To be detected, the radical pair must escape from the reaction cage to avoid fast back electron transfer. Because thionine binds intimately to DNA via intercalation,[21] the back electron transfer is likely to be faster than dissociation of the reduced thionine. Because no cage escape occurs, no long-lived transient is observed. Thus, the lack of DNA damage occurs despite guanine oxidation by excited thionine because the rapid back electron transfer does not permit any net reaction.

Here, we have shown that photoexcited thionine oxidizes the guanine base but that this process does not lead to oxidative damage in duplex DNA. Trapping of guanine radical to produce alkali-labile sites is slow as compared to electron transfer through or with DNA bases. Hence, the rate at which guanine radical is reduced (e.g., back electron transfer) can affect both the amount and the distribution of permanent guanine damage. We recently showed that the presence of an efficient reductant at the site of hole injection substantially diminishes the propagation of charge out to a distant DNA site.[22] Indeed, in the case of thionine, the rapid back electron transfer from semithionine radical to guanine radical does not allow for any permanent damage. As this extreme example shows, then, yields of oxidative damage do not necessarily reflect the rate of radical formation and charge transport in DNA. Just as charge recombination limits the efficiency of photosynthesis, so too does it govern DNA charge transport damage. Although a compound may bind DNA with high affinity and possess a redox potential suitable for base oxidation, this may not be sufficient to cause permanent DNA damage.

Acknowledgment. We are grateful to the NIH for financial support (GM49216). C.D. also thanks the JSPS for a postdoctoral fellowship.

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(19) This potential measured for thionine in water may not be identical to the potential of DNA-bound thionine but is certainly sufficient to oxidize guanine (1.3 V).[1a]
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JA036397Z