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Photo-Oxidation of Duplex DNA with the Stable Trioxatriangulenium Ion

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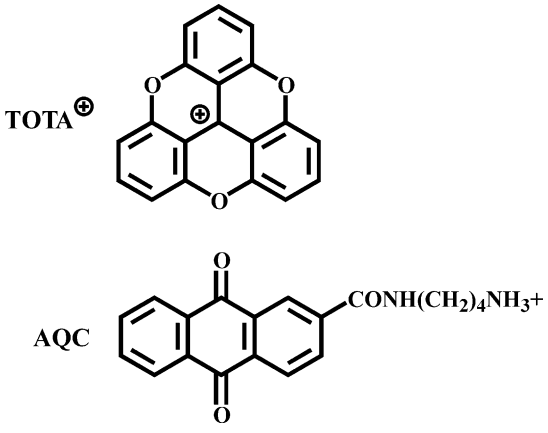
Abstract—We show that 5′-Gs in 5′-GG-3′ duplex DNA dinucleotide steps are preferentially oxidized by the trioxatriangulenium ion (**TOTA**$^{\oplus}$) upon photo-activation, an oxidation pattern characteristic of guanine radical cation formation. Some photo-oxidation of the 3′G in 5′GG3′ steps and of isolated guanines is also observed but reactions carried out in D_2O reveal only a minor increase in oxidation damage at these sites, indicating that electron transfer is the primary mechanism of guanine oxidation.

The trioxatriangulenium ion, **TOTA**$^{\oplus}$, is a stable planar carbocation. Direct evidence for the planarity of **TOTA**$^{\oplus}$ was recently obtained by Krebs et al. from an X-ray crystallographic investigation of 10 salts derived from **TOTA**$^{\oplus}$.[1] This study revealed that all the core atoms of **TOTA**$^{\oplus}$ are co-planar. Bond lengths between the central carbon atom and the three aromatic ring carbon atoms to which the central carbon atom is attached were found to be typical of aromatic systems (1.384–1.409 Å). Ab initio and density functional theory (DFT) methods were recently used to determine vibrational spectra and optimized geometry of **TOTA**$^{\oplus}$.[2] The theoretical results were then compared to the experimentally obtained spectra and the X-ray crystal structure. These calculations have shown that the positive charge in **TOTA**$^{\oplus}$ is substantially delocalized.[2] If the positive charge is shared by the oxygen atoms, **TOTA**$^{\oplus}$ may show properties similar to those of pyrylium and xanthelium salts, which are good acceptors of electrons in their excited states.[3,4]



We have observed that the fluorescence of **TOTA**$^{\oplus}$ is quenched by several aromatic hydrocarbons, halogen compounds, ethers, and amines in acetonitrile solutions.[5] In general, fluorescence quenching occurs due to energy transfer, electron transfer, exciplex formation or excited state reactions. For energy transfer to occur efficiently, the absorption band of the quencher molecules should overlap with the fluorescence band of **TOTA**$^{\oplus}$. However, all the quencher molecules we employed have absorptions at lower wavelengths compared to **TOTA**$^{\oplus}$, and hence the occurrence of energy transfer can be ruled out in these cases. Exciplex formation usually occurs in non-polar solvents and is

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accompanied by the formation of emission bands at longer wavelengths.[6,7] In our experiments, we have not observed any long wavelength emission bands, even at the highest quencher concentrations employed, thus ruling out exciplex formation.[8] If fluorescence quenching is due to excited state reactions, then prolonged irradiation in the presence of the quencher should result in the loss of absorption intensity of **TOTA**$^{\oplus}$ and formation of absorption bands due to the product. Since the absorption spectrum of the **TOTA**$^{\oplus}$/quencher systems did not exhibit any change, even after prolonged irradiations, excited state reactions were also ruled out.[8] Thus, the fluorescence quenching observed is most likely due to an electron transfer process.

The quenching rate constants for the various electron donors mentioned above were obtained by the usual Stern–Volmer method and we have shown that the quenching rate constants thus obtained followed the Rehm–Weller behavior.[5] This is further proof that quenching is due to electron transfer. We have further confirmed electron transfer by flash photolysis experiments. Spectra corresponding to the radical cation of the donor and radical of **TOTA** were obtained in these studies.[5,8] Fluorescence of **TOTA**$^{\oplus}$ is also quenched by guanosine through an electron transfer mechanism.[5] In flash photolysis experiments we have observed the **TOTA** radical;[8] however we have been unable to observe the guanine radical cation, most probably due to its low extinction coefficient.[9]

The stability of **TOTA**$^{\oplus}$ and its efficiency as an electron acceptor upon photo-activation indicates that it should serve as an excellent probe for charge transfer in DNA. Its planar aromatic structure is ideally suited for intercalation into duplex DNA and equilibrium dialysis studies have shown that **TOTA**$^{\oplus}$ binds readily to duplex DNA, with some preference for GC base pairs.[10] We show here that **TOTA**$^{\oplus}$ readily oxidizes duplex DNA upon photo-activation and that the preferred sites of oxidation are 5′-G residues in 5′-GG-3′ dinucleotide steps. This is a characteristic oxidation pattern that has been ascribed to favorable stacking interactions that render the 5′-G residue in 5′-GG-3′ steps a better sink for the electron hole generated in DNA as a result of electron transfer.[11] We also observed some oxidation of the 3′Gs in 5′-GG-3′ steps, as well as slight oxidation of isolated G residues, likely as a result of some singlet oxygen generation. However, photo-oxidation of guanine residues by **TOTA**$^{\oplus}$ does not increase significantly in D_2O solutions, indicating that oxidation through singlet oxygen is not as significant as oxidation through electron transfer. **TOTA**$^{\oplus}$ was further compared with the known anthraquinone-based photo-oxidizer *N*-(4-aminobutyl)-2-anthraquinonecarboxamide hydrochloride (**AQC**)[12–16] Comparison of the DNA fragments following piperidine heat-treament under identical conditions showed similar cleavage patterns for both **TOTA**$^{\oplus}$ and **AQC**.

We examined the pattern of guanine oxidation by **TOTA**$^{\oplus}$ and **AQC** in duplex DNA using the substrate 5′-TACTTGTTGTTGGTTTGGTTTGTTGTTGTT-3′.5′-AACAACAACAAACCAAACCAACAACAAGTA-3′ labeled with γ-^{32}P on the G-containing strand. Photo-oxidation of duplex DNA is known to lead to base-labile oxidation products that can be revealed by treatment of the DNA with hot piperidine.[17–19] Lane 5 in Figure 1A shows the cleavage products obtained for the G-containing strand 5′-TACTTGTTGTTGGTTTGGTTTGTTGTTGTT-3′ after irradiation (350 nm, 30 min) in the presence of 5 μM **TOTA**$^{\oplus}$ in H_2O.[20] The corresponding histogram in Figure 2A shows a clear preference for oxidation of 5′guanines in the two 5′-GG-3′ dinucleotide steps. This type of oxidation pattern is a hallmark of oxidation via electron transfer.[17–19]

In principle, excitation of **TOTA**$^{\oplus}$ in solutions not oxygen-free can lead to generation of singlet oxygen, which can also oxidize guanine residues.[18] The quantum yield of triplet formation for **TOTA**$^{\oplus}$ is about 0.6 in aqueous solutions[5] and thus singlet oxygen quantum yield could

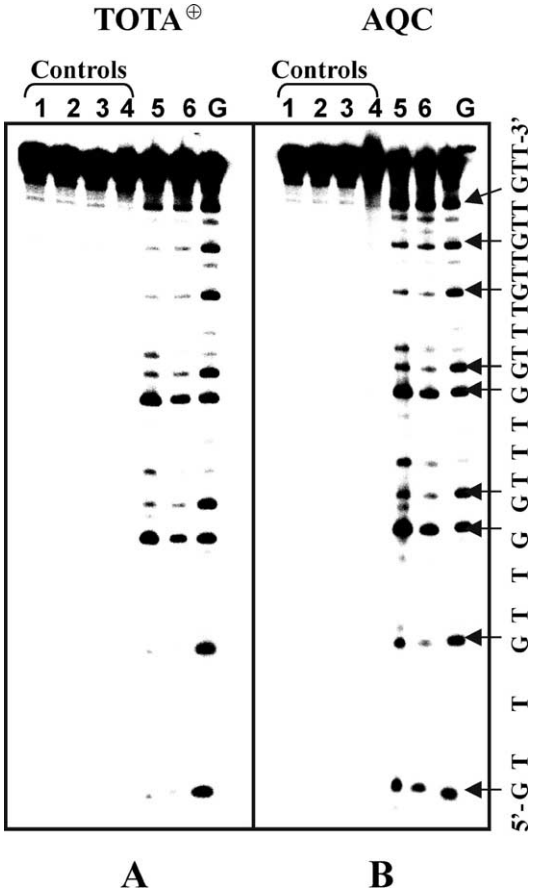

Figure 1. (A) Phosphorimage of the cleavage pattern observed for the G-containing strand in the duplex 5′ - TACTTGTTGTTGGTTTGGTTTGTTGTTGTT - 3′.5′ - AACAACAACAAACCAAACCAACAACAAGTA-3′ after irradiation (350 nm, 30 min) in the presence of 5 micromolar **TOTA**$^{\oplus}$ in H_2O/D_2O sodium phosphate buffer, pH 7.0. Lanes 1–5 (in A and B) correspond to reactions performed in H_2O. Lane 1: DNA plus **TOTA**$^{\oplus}$ but no light irradiation; lane 2: DNA without **TOTA**$^{\oplus}$ but irradiated; lane 3: DNA without **TOTA**$^{\oplus}$ and no irradiation; lane 4: DNA irradiated in the presence of **TOTA**$^{\oplus}$ but not treated with hot piperidine; lane 5: DNA irradiated in the presence of **TOTA**$^{\oplus}$ and treated with piperidine; lane 6: DNA irradiated in the presence of **TOTA**$^{\oplus}$ in D_2O and treated with piperidine; lane G is a Maxam–Gilbert sequencing lane. (B) Same as in (A) but irradiated in the presence of **AQC**.

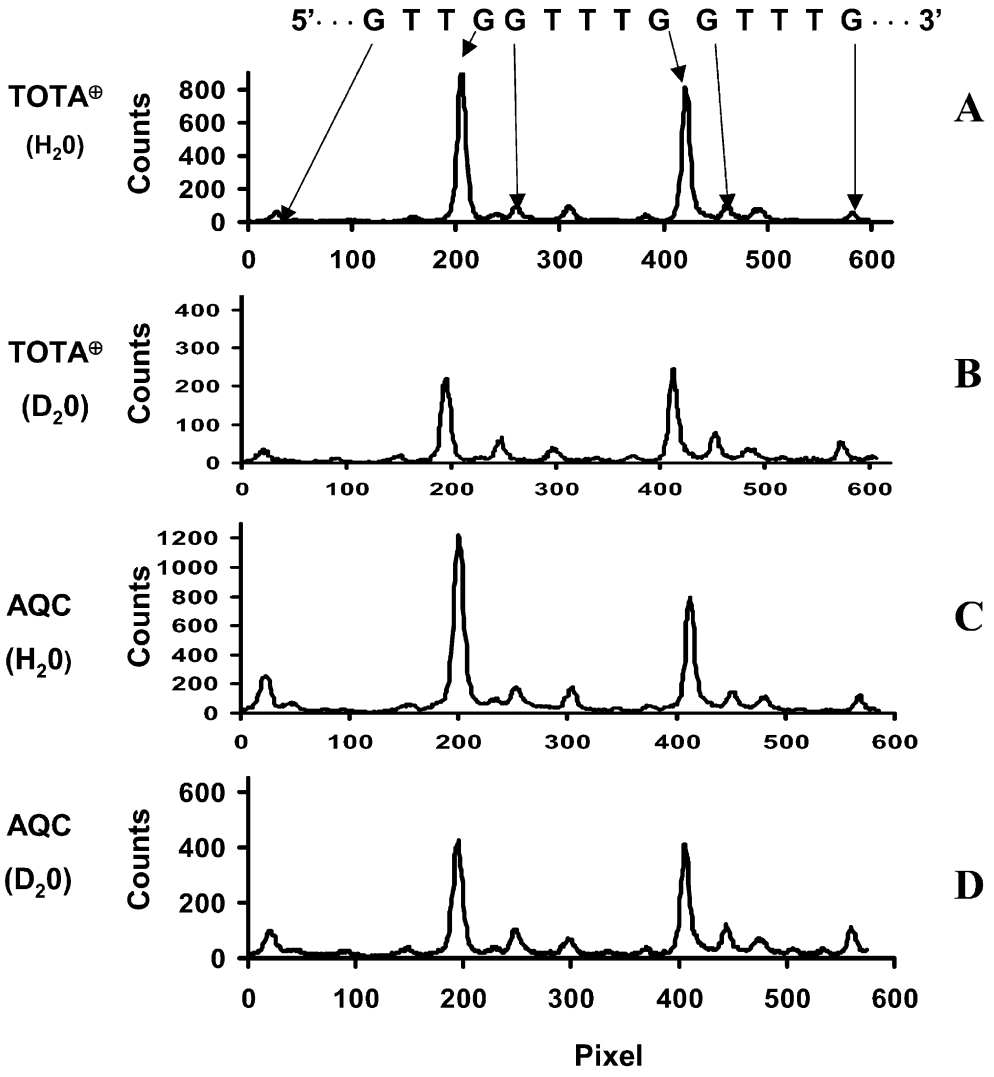

Figure 2. (A) and (B) Histograms of lanes 5 and 6, respectively, from the denaturing polyacrylamide gel shown in Figure 1A corresponding to photo-oxidation reactions in H_2O (lane 5) and D_2O (lane 6) for **TOTA**$^{\oplus}$. (C) and (D) are histograms of lanes 5 and 6, respectively, from the denaturing polyacrylamide gel shown in Figure 1B corresponding to photo-oxidation reactions in H_2O (lane 5) and D_2O (lane 6) for **AQC**.

be as high as 0.6. Guanine oxidation via singlet oxygen is known to be non-specific and likely contributes to the observed (although rather minor) oxidation of the isolated G residues in the sequence, as well as oxidation of the 3′Gs in the two 5′-GG-3′ steps. To examine the extent of oxidation arising from singlet oxygen, we repeated the same photo-oxidation experiment with **TOTA**$^{\oplus}$ but in D_2O solution. Because the lifetime of singlet oxygen is increased by a factor of about 13 in D_2O,[21] running the reactions in D_2O should result in more efficient oxidation of guanine residues relative to that observed in H_2O. Lane 6 in Figure 1A shows the cleavage products obtained for the G-containing strand 5′-TACTTGTTGTTGGTTTGGTTTGTTGTTGTT-3′ under the same experimental conditions as in lane 5, Figure 1A, except for D_2O. The corresponding histogram in Figure 2B shows that preferential cleavage of 5′-Gs in the two 5′-GG-3′ dinucleotide steps is conserved in D_2O. Furthermore, oxidation of the two isolated guanines towards the 5′-end of the DNA strand, although still evident, is not noticeably enhanced in D_2O versus H_2O. Thus, oxidation through singlet oxygen, while still a possibility for **TOTA**$^{\oplus}$, appears to be less likely than oxidation through electron transfer.

The results in lanes 5 and 6 of Figure 1A and the corresponding histograms in Figure 2A and B show that guanine residues are generally less effectively oxidized in D_2O than in H_2O; an effect that is more pronounced for the 5′Gs in the two 5′-GG-3′ steps. The reduced oxidation of guanines in D_2O can be ascribed to a kinetic deuterium isotope effect, in which either the reactivity of the guanine radical cation towards water (to yield 8-oxoguanine), or deprotonation of the guanine radical cation (which then yields an oxazolone lesion after reaction with oxygen), is reduced.[22–24]

Figure 1B shows the hot piperidine-induced cleavage products observed after photo-oxidation of the same DNA strand with the known photo-oxidizer **AQC**.[12–15] The experiments were performed under identical conditions as with **TOTA**$^{\oplus}$, including the same photo-oxidizer concentration. Lane 5 in Figure 1B and the corresponding histogram in Figure 2C show that, while

AQC also preferentially oxidizes 5′G residues in 5′-GG-3′ dinucleotide steps, it leads, however, to more oxidation of isolated G residues compared to **TOTA**$^{\oplus}$. Lane 6 in Figure 1B and the corresponding histogram in Figure 2D show that oxidation of isolated G residues and 3′G residues in the two 5′-GG-3′ dinucleotide steps also persists in D_2O and that the products of oxidation are also generally reduced, as observed with **TOTA**$^{\oplus}$. Lanes 4 in Figure 1A and B show that there is no noticeable spontaneous non-selective cleavage for either **TOTA**$^{\oplus}$ or without hot piperidine-treatment under these conditions.

From a comparison of the data in Figures 1 and 2, the extent of 5′G cleavage (in 5′-GG-3′ dinucleotide steps) compared to cleavage of other G residues in this DNA is somewhat greater for **TOTA**$^{\oplus}$ than for **AQC**, despite the fact that the extinction coefficient, ε_{350}, of **TOTA**$^{\oplus}$ is nearly twice that of **AQC**. Thus, while oxidation through singlet oxygen is likely to contribute to the photo-oxidation products observed with **TOTA**$^{\oplus}$, the data indicate that **TOTA**$^{\oplus}$ is an effective electron transfer agent. The formation of a guanine radical cation, typical of guanine oxidation via electron transfer, is supported for both **TOTA**$^{\oplus}$ and **AQC** by the preferred oxidation of 5′Gs in 5′-GG-3′ steps, and by the fact that oxidation of 5′Gs in 5′-GG-3′ dinucleotide steps is significantly reduced in D_2O.

In conclusion, despite the observed relatively minor oxidation of isolated G residues, which likely arises from singlet oxygen, the stable carbocation, **TOTA**$^{\oplus}$, is an effective electron transfer agent comparable to the known photo-oxidant **AQC**, which has been reported to oxidize DNA through an electron transfer mechanism[12–15] under similar conditions as those used here. The differences in the cleavage intensity patterns observed for **TOTA**$^{\oplus}$ versus **AQC** could, in principle, be due to different binding constants. However, the data show that **TOTA**$^{\oplus}$ should prove a valuable addition to the chemical library of electron transfer agents that can be used to study charge transport in DNA in a controlled fashion. The data suggest an intercalation mode for the binding of **TOTA**$^{\oplus}$ to DNA since this type of interaction is necessary for efficient electron transfer to occur. Further studies to determine the exact mode of binding to DNA are under way.

Acknowledgements

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20. 50-μM stock solutions of the perchlorate salt of **TOTA**$^{\oplus}$ or the hydrochloride salt of **AQC** were prepared in water solutions containing 0.25% v/v DMSO. The DNA duplexes were prepared and labeled with [γ^{32}P]ATP using standard procedures. The reactions were set up in 20 μL volume reaction mixtures consisting of DNA substrate (30,000 cpm, ~300 fmol), 100 mM KCl, and 5 μM of either TOTA$^{\oplus}$ or **AQC** in a 50-mM sodium phosphate buffer (pH 7.0). The tubes were irradiated in an LZC-4 photoreactor (Luzchem) at 350 nm for 30 min. The reactions were stopped by adding stop buffer consisting of 0.1 M thiourea, 0.3 M sodium acetate (pH 5.2) and 10 μg tRNA. The samples were then subjected to ethanol precipitation. After pelleting the DNA, samples were washed with 70% ethanol and dried. These samples were then subjected to piperidine heat treatment (1 M piperidine, 90 °C, 15 min). The cleaved products were finally resolved on 16% denaturing PAGE. The experiments in D_2O were performed in identical fashion, except that the samples were lyophilized at least four times from D_2O and finally redissolved in D_2O before irradiation with light.
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