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Synthesis and Study of Naphthacenedione (TQ) as a Photosensitizer for One-Electron Oxidation of DNA

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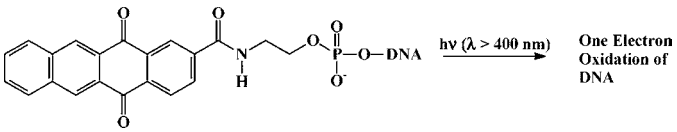
ABSTRACT



Photosensitizers are useful for the study of one-electron oxidation of DNA. Most such photosensitizers absorb light in the UV spectral region. We report the synthesis and investigation of a 5,12-naphthacenedione (TQ) derivative as a DNA photosensitizer. Irradiation of a TQ-linked duplex with visible light results in reaction of the DNA that is characteristic of one-electron oxidation. The results from TQ sensitization are identical, within experimental error, with that of a well-studied anthraquinone derivative.

The photochemical oxidation of DNA is a topic of widespread interest because of its relevance to the survival of cellular organisms and because of its possible application to molecular electronics.[1] A key component in the study of light-induced one-electron oxidation of DNA is the sensitizer. The sensitizer absorbs the activating light and while in its excited state receives an electron from a DNA nucleobase. This electron transfer reaction creates a base radical cation ("hole") and converts the sensitizer to its radical anion. The ground state neutral sensitizer is eventually regenerated by loss of an electron from the radical anion. This may occur by charge annihilation with the base radical cation, which results in no net reaction, or by reduction of another species, which often is molecular oxygen that is converted to superoxide. The latter process yields a base radical cation isolated in the DNA that may migrate through the duplex until it is eventually trapped irreversibly by reaction with H_2O or O_2 forming a "lesion".[2]

Innumerable compounds have been tested for their ability to sensitize the one-electron oxidation of DNA.[3−5] Among the most useful are those that are covalently linked to the DNA. In these cases, the site of the initial one-electron oxidation is defined by the chemical structure. One of the most valuable of the covalently linked sensitizers is based on the anthraquinone chromophore (AQ).[5] Excitation of AQ forms a singlet excited state that intersystem crosses to the triplet very rapidly. The triplet AQ is thermodynamically capable of oxidizing all of the four common DNA nucleobases to their radical cations. As a consequence, the radical ion pair that results ($AQ^{-\bullet}B^{+\bullet}$) is in an overall triplet state, and this inhibits the energy-wasting back electron-transfer reaction.

The AQ absorption spectrum extends to ca. 350 nm, which is convenient because it permits excitation of AQ without simultaneous absorption by the DNA. However, there are circumstances when it is desirable to oxidize DNA in the presence of a biological component or drug that absorbs in the UV region of the spectrum. In these cases, it is necessary

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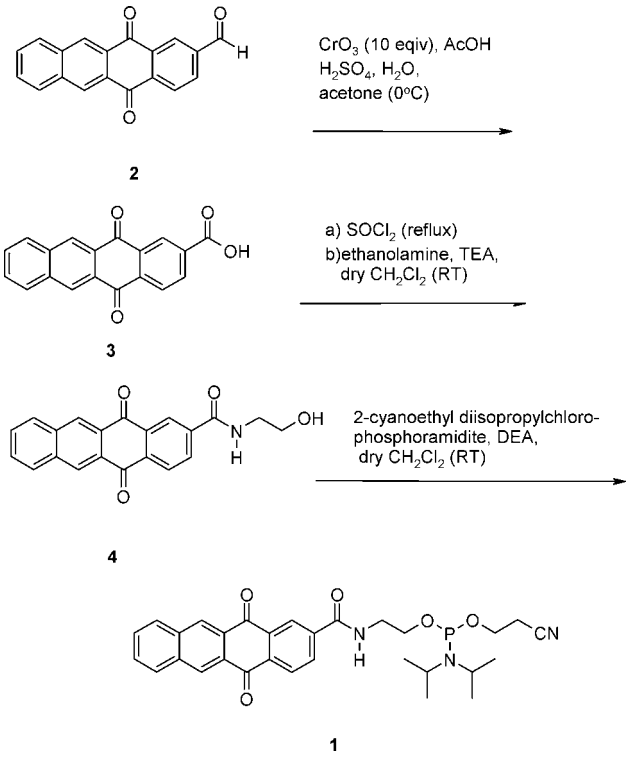
to utilize a sensitizer having photophysical and electrochemical properties similar to those of AQ but with an absorption spectrum that extends into the visible region. We report here the development of a naphthacenedione (tetracenequinone, TQ)-based DNA sensitizer (Scheme 1) and demonstrate its value in the one-electron oxidation of DNA with visible light.

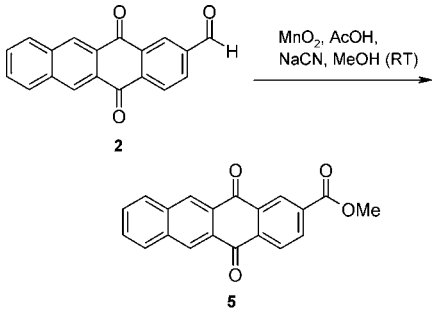
Scheme 1. Synthetic Method for Preparation of Phosphoramidite **1**



The objective of our synthetic work is TQ-substituted phosphoramidite **1**; see Scheme 1. This compound can be incorporated covalently at the 5′-terminus of DNA oligomers prepared by automated synthesis. Our synthesis of **1** began with the known TQ carboxaldehyde **2**.[6−8] This aldehyde was converted to carboxylic acid (**3**) in 49% yield by oxidation with CrO_3 in a 1:1 acetic acid/acetone solution. The acid was activated by reaction with thionyl chloride to form the acid chloride, which was used immediately in subsequent reactions without isolation. The TQ acid chloride reacts well with ethanolamine in a dry methylene chloride solution containing triethylamine to give TQ-amide **4** in a 55% yield after recrystallization. Amide **4** is readily converted to **1** by reaction with 2-cyanoethyl diisopropylchlorophosphoramidite in dry methylene chloride solution containing diisopropylethylamine. Upon purification by silica gel column chromatography, which gave a 95% yield, **1** was suitable for use in automated DNA synthesis. Detailed experimental procedures are presented in Supporting Information.

We required a soluble, stable, model TQ-containing compound to study the photophysical and electrochemical properties of this chromophore. We prepared TQ methyl ester **5**[9] for this purpose in 83% yield by oxidation of a methanol/acetic acid solution of aldehyde **2** with MnO_2 in the presence of NaCN as is shown in Scheme 2.

Scheme 2. Synthetic Procedure for Preparation of TQ Methyl Ester **5**



We assessed the chemical, electrochemical, and photophysical properties of the TQ chromophore to determine its suitability as a light-induced sensitizer of DNA oxidation. The absorption spectrum of the TQ chromophore in acetonitrile extends into the visible region with a peak at 400 nm and an extinction coefficient at this maximum of 4200 M^{-1} cm^{-1}. These properties indicate that the TQ chromophore may be suitable as a sensitizer for visible-light-induced oxidation of DNA.

Electron transfer reactions can occur rapidly when they are exothermic. The free energy of a light-induced one-electron oxidation (ΔG_{ET}) can be estimated by application of the Weller equation (eq 1),[10] where E_{red} is the reduction potential of the electron acceptor (the TQ in this case), E_{ox} is the oxidation potential of the electron donor (a nucleobase in this case), E^* is the energy of the relevant excited state of the sensitizer (the triplet of TQ in this case), and E_{work} is a term that reflects the work required to separate charge in the dielectric of the solvent.

$$\Delta G_{ET} = E_{ox} - E_{red} - E^* + E_{work} \qquad (1)$$

The excited-state energy, $E^{3}*$, of the triplet TQ chromophore was determined by measuring the phosphorescence spectrum of the ester **5** in an ethanol glass at liquid nitrogen temperature. The $0 \rightarrow 0$ band of the emission was assigned at 492 nm, which corresponds to a triplet energy of 2.37 eV. No fluorescence is detected in this experiment, which indicates, as expected, that intersystem crossing of TQ is rapid.

The reduction potential of the TQ chromophore was determined by cyclic voltammetry of ester **5** in an N_2-

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saturated acetonitrile solution containing 0.1 M tetrabutylammonium hexafluorophosphate. A three-electrode system consisting of a platinum working electrode, a Ag/AgCl reference electrode, and a platinum auxiliary electrode was used to measure the reduction potential. The voltammogram showed a reversible reduction wave at −0.88 V vs NHE (corrected from Ag/AgCl by reference with ferrocene).[11]

The value of ΔG_{ET} for electron transfer from each of the four nucleobases to the electronically excited triplet TQ chromophore was estimated from the measured values of E_{red}, E^{*3}, and reported values for E_{ox} of the nucleobases.[12] Water is the relevant solvent for these experiments, and thus the Coulombic work term makes a negligible contribution to ΔG_{ET} because of its high dielectric constant. The estimates of ΔG_{ET} are summarized in Table 1. The ΔG_{ET} values for oxidation of C and T are positive, which indicates an unfavorable free energy for charge transfer between triplet TQ and the pyrimidines. However, ΔG_{ET} values for oxidation of the purines (guanine and adenine) by triplet TQ are negative, which indicates that triplet TQ may be a good electron-transfer sensitizer for DNA because each base pair contains one purine and one pyrimidine.

Table 1. Calculated Gibbs Free Energy for the four DNA Bases According to the Weller Equation

bases	E_{ox} (V) vs NHE[a]	ΔG_{ET} (eV)
guanosine	1.29	−0.26
adenosine	1.42	−0.13
cytidine	1.6	0.05
thymidine	1.7	0.15

[a] Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617−618.

A TQ-linked DNA duplex was prepared to test the utility of TQ as a photosensitizer. The TQ-phosphoramidite (**1**) was incorporated in the last step (5′-terminus) of the automated synthesis of DNA oligomer S1, which is one of the two strands of duplex DNA(1); see Figure 1. The TQ-phosphoramidite incorporation typically occurs with an efficiency of ca. 65%, which is similar to results obtained with AQ phosphoramidite. The deprotected oligomer S1 was purified and analyzed by HPLC, which showed the presence of only one compound. The mass of the oligomer was confirmed by MALDI-TOF mass spectroscopy, which showed a molecular ion peak at *m*/*e* 8709 (calculated *m*/*e* 8710) confirming the success of the synthesis. The UV spectrum of S1 is particularly revealing; see Figure 2. In addition to the usual DNA absorption band with a maximum at ca. 260 nm, it exhibits a visible absorption band for the TQ chromophore at 420 nm, which is a spectral region where DNA is normally transparent. In acetonitrile solution, TQ ester **5** exhibits a maximum in the absorption spectrum at 400 nm. The red shift of the TQ absorption from 400 to 420 nm may be a consequence in part of the interaction of the TQ chromophore with the aromatic nucleobases of the DNA and in part an effect of the solvent change.

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TQ-5′-AAA TG CC  GGTAC AAA CATGG CC  GT ACG -3′ (S1)
   3′-TTT AC(GG_p)CCATG TTT GTACC(GG_d)CA TGC*-5′ (S2)
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Figure 1. TQ-linked DNA(1). The ∗ indicates the position of the ^{32}P radiolabel. AQ-linked DNA(1) is identical except that an anthraquinone chromophore replaces the tetracenequinone.

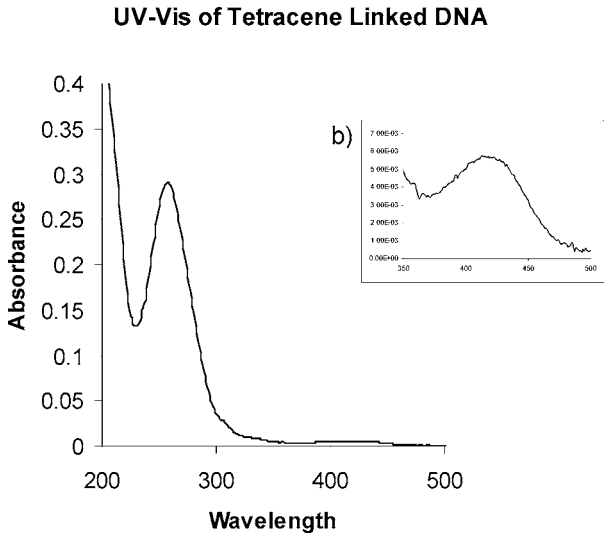

Figure 2. UV spectrum of TQ-DNA strand S1 in H_2O solution displaying the characteristic absorption of DNA and the TQ absorbance at 420 nm. (b) Expansion of the region from 350 to 500 nm of the TQ-DNA spectrum showing more clearly the absorption maximum at ∼420 nm characteristic of TQ.

Duplex DNA(1) was prepared by hybridizing S1 with its complementary strand S2, which was similarly prepared, purified, and analyzed. The duplex shows the characteristic circular dichroism (CD) spectrum of B-form DNA with a positive maximum at 282 nm. The melting behavior of DNA(1) is also as expected. A 10 mM sodium phosphate buffer (∼pH 7.0) solution of DNA(1) exhibits a cooperative melting transition (T_m) at 62 °C, which shows that this duplex will be stable under the conditions of the irradiation experiment (30 °C). The T_m of an AQ-linked DNA oligomer of the same sequence is 61 °C, which shows that the AQ and TQ sensitizers interact similarly with the DNA.

Experiments with various sensitizers under a range of conditions have revealed a characteristic of the one-electron oxidation of duplex DNA to be reaction primarily at the 5′-G of GG steps.[3] This reaction typically is revealed as strand cleavage by polyacrylamide gel electrophoresis (PAGE) after treatment of the oxidized DNA with piperidine. PAGE generally requires that one strand of the DNA be labeled

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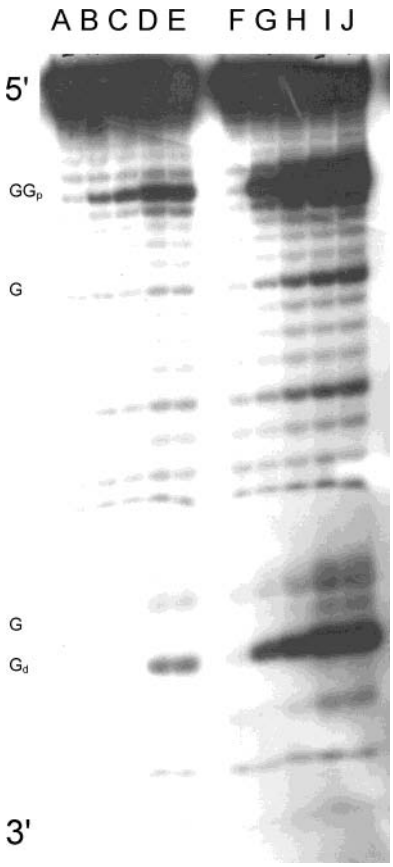

Figure 3. Autoradiogram of TQ-linked DNA(1) and AQ-linked DNA(1). Lane A is the dark control (without irradiation) of TQ-DNA. Lanes B, C, D, and E are the experimental lanes for TQ-DNA irradiated for 2, 4, 10, and 20 min, respectively, at wavelengths greater than 400 nm with a 150-W Xe arc lamp. Lanes F−J are those for AQ-DNA. Lane F is the dark control. Lanes G, H, I, and J are the experimental lanes for AQ-DNA samples irradiated for 2, 4, 10 and 20 min, respectively, in a Rayonet reactor equipped with eight 24-W 350 nm lamps. The data in lanes I and J are outside of single hit conditions and were not used in the analysis. Because different lamps and irradiation geometry were used for the AQ- and TQ-DNA, it is not possible to compare efficiencies directly.

with a radioisotope to allow analysis by autoradiography and phosphorimagery. We incorporated ^{32}P (the ∗ in Figure 1) at the 5′-terminus of S1 for this purpose.[13] Strand S1 contains two GG steps. The one closer to the TQ that we refer to as proximal (GG_p) is seven base pairs, ca. 24 Å, from the TQ and the second (distal step) GG_d is ca. 61 Å from the TQ.

Samples of radiolabeled DNA(1) (10 000 cpm) in aqueous sodium phosphate buffer solution were irradiated at room temperature at wavelengths greater than 400 nm with a 150-W Xe lamp for 2, 4, 10, and 20 min. The irradiated samples, along with a dark control (sample not exposed to light), were treated with piperidine, analyzed by PAGE, and visualized by autoradiography; see Figure 3. Strand cleavage is readily detected at the 5′-G of the proximal and distal GG steps of S2, which indicates that the TQ chromophore is capable of sensitizing the one electron oxidation of DNA.

A typical quantitative assessment of the distance dependence of radical cation migration in DNA is the ratio of strand cleavage at the proximal and distal GG steps. Ideally, this ratio should be independent of the identity of the sensitizer.[14] We compared the ratio of proximal to distal cleavage obtained from irradiation of DNA(1) at >400 nm with an identical oligomer substituted with the usual AQ sensitizer irradiated at 350 nm for 2, 4, 10, and 20 min. The results are shown in Figure 3; the ratio of proximal to distal damage obtained from sensitization with TQ is indistinguishable from that observed with AQ sensitization.

In summary, we have shown that the TQ chromophore is a suitable photosensitizer for the one-electron oxidation of duplex DNA that permits experiments to be carried out with irradiation at wavelengths greater than 400 nm.

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Supporting Information Available: Cyclic voltammerty results for TQ ester **5**; TQ-DNA melting behavior; NMR spectra of TQ ester **5** and phosphoramidite **1**; and detailed descriptions of experimental procedures for the synthesis of TQ phosphoramidite **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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