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Anthraquinone Photonucleases: Mechanisms for GG-Selective and Nonselective Cleavage of Double-Stranded DNA

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Abstract: Anthraquinone derivatives 2AQA2(HEt_2) and 2AQC2(HEt_2) were examined as light-activated agents that initiate DNA cleavage. The substituents control the electronic configuration of the lowest excited state of the anthraquinone. 2AQC2(HEt_2) has a lowest $n\pi^*$ excited state and can react by electron transfer or hydrogen atom abstraction. 2AQC2(HEt_2) has a lowest excited state of $\pi\pi^*$ or intramolecular charge-transfer character and reacts only by electron transfer. Spectroscopic evidence indicates that both quinones bind to double-stranded DNA by intercalation with essentially the same affinity. Picosecond time-resolved laser spectroscopy shows that single electron transfer from the DNA bases to either bound quinone occurs rapidly and to the same extent. Irradiation of either intercalated 2AQA2(HEt_2) or 2AQC2(HEt_2) followed by treatment with hot piperdine leads to equally effective cleavage of DNA at the 5′-G of GG steps. These findings indicate that electron transfer from a DNA base to the excited quinone is the dominant path for the GG-selective DNA cleavage. At high concentrations, where some quinone is free in solution, irradiation of 2AQC2(HEt_2), but not 2AQA2(HEt_2), leads to nonselective spontaneous cleavage of DNA. This second path to DNA cleavage is identified as direct hydrogen atom abstraction from the deoxyribose backbone by excited, nonintercalated 2AQC2(HEt_2).

Introduction

The design and investigation of artificial nucleases,[1] small molecules capable of cleaving duplex or single-stranded DNA, is an area of exciting research that has numerous important biochemical and biomedical applications. While most of these agents are activated thermally, there is an increasing emphasis on photoactivated cleavage agents[2] since this methodology possesses significant practical advantages. In particular, photonucleases can be triggered by exposure to light. Light is an attractive cofactor since it is easy to manipulate.

We previously reported the efficient nicking of supercoiled plasmid DNA by water-soluble, UV-light-activated anthraquinone derivatives.[3] These photonucleases are catalytic, being regenerated by reaction with oxygen, and therefore may initiate coincident site double-strand cleavage of DNA.[4] Absorption and circular dichroism spectra indicate that some of these anthraquinone derivatives form intercalative complexes with

[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1996.

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(3) Koch, T.; Ropp, J. D.; Sligar, S. G.; Schuster, G. B. *Photochem. Photobiol.* **1993**, *58*, 554−558.

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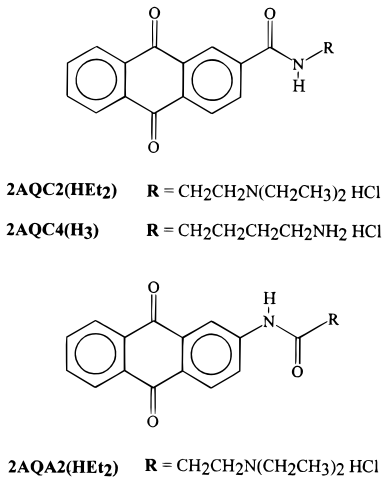
Chart 1



DNA. Time-resolved laser spectroscopic studies indicated that two processes are initiated when the anthraquinone−DNA complexes are irradiated: (*i*) photoinduced electron transfer from a neighboring nucleic acid base to the excited anthraquinone which was directly detected and (*ii*) hydrogen atom abstraction from a deoxyribose unit which was inferred by analysis of the decay kinetics.[4] DNA cleavage can occur from either pathway, and the initial spectroscopic observations were interpreted to indicate that the hydrogen atom transfer route was the most effective.

Further investigation has revealed that these monosubstituted anthraquinone derivatives cleave double-stranded DNA selectively at the 5′-G of a GG step.[5] Very recently, photonuclease activity with similar base-pair selectivity has been reported for three other compounds including two naphthalimide derivatives[6] and riboflavin, a naturally occurring imide.[7] This pattern of selective DNA cleavage is attributed to electron-transfer reactions initiated by an electronically excited state.[6a]

Anthraquinone derivatives 2AQA2(HEt_2) and AQC2(HEt_2) (Chart 1)[8] were examined to provide insight into the reaction mechanism of the excited quinones with DNA that leads to strand cleavage. The excited states of both 2AQA2(HEt_2) and 2AQC2(HEt_2) are capable of the single electron oxidation of purine and pyrimidine bases, but only 2AQC2(HEt_2) is expected to efficiently abstract a hydrogen atom. This investigation reveals that electron transfer is the dominant pathway for the GG-selective cleavage of DNA by intercalated anthraquinone[9] and that nonsequence-selective spontaneous cleavage of DNA results from reaction with nonintercalated quinone.

Results

(1) Photochemical and Photophysical Properties of 2AQA2(HEt_2) and 2AQC2(HEt_2). One of the hallmarks of carbonyl group photochemistry is how dependent some properties are on the electronic configuration of the lowest energy excited state.

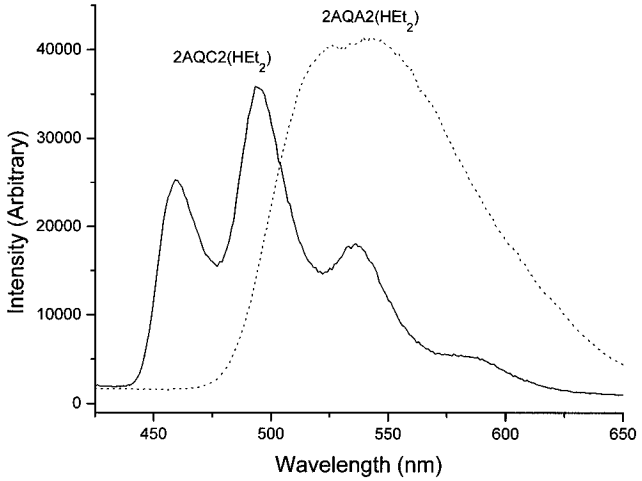


Figure 1. Phosphorescence spectra of 2AQA2(HEt_2) and 2AQC2(HEt_2) recorded at 77 K in a 75% PBS−25% glycerol and ethanol, respectively. The emission spectra have been normalized.

For example, ketones that have predominant nπ^* character generally intersystem cross to triplets with high rates, abstract hydrogen atoms rapidly, and have structured phosphorescence spectra. In contrast, ketones with predominant $\pi\pi^*$ or charge-transfer character often fluoresce in fluid solution, do not react rapidly with hydrogen atom donors, and have structureless phosphorescence spectra. Significantly, the rates of electron-transfer reactions of excited ketones depend primarily on energetic factors and are essentially independent of the specific electronic configuration of the state. We examined the spectral properties and excited state reactivity of 2AQA2(HEt_2) and 2AQC2(HEt_2) to assess the electronic configuration of their excited states.

(a) Absorption and Luminescence of 2AQA2(HEt_2) and 2AQC2(HEt_2). A strong indication of the distinct electronic characteristics of 2AQA2(HEt_2) and 2AQC2(HEt_2) is found in their absorption spectra. In aqueous solutions, 2AQC2(HEt_2) absorbs with $\lambda_{max} = 320$ nm, which is identical with that of unsubstituted anthraquinone. In contrast, the absorption maximum of 2AQA2(HEt_2) is found at 375 nm with tailing into the visible region. This 55 nm bathochromic spectral shift in absorption maximum clearly signals the participation of low lying charge-transfer excited states in 2AQA2(HEt_2).

The emission spectra of 2AQA2(HEt_2) and 2AQC2(HEt_2) are also dramatically different. 2AQC2(HEt_2) is not emissive at room temperature in an aqueous PBS buffer solution, but under identical conditions, 2AQA2(HEt_2) fluoresces, with $\phi_{fl} = 0.02$ and a lifetime of 1.2 ns. At 77 °K, the emission spectrum of 2AQA2(HEt_2) broadens to include a phosphorescence component with a lifetime of 44 ± 8 μs (in an ethanol glass) (Figure 1), but neither the fluorescence or phosphorescence shows any vibronic structure. The unstructured emission of 2AQA2(HEt_2) is typical of a ketone triplet with a lowest energy $\pi\pi^*$ or charge-transfer excited state. Strong phosphorescence of 2AQC2(HEt_2) is observed in a frozen 75% PBS−25% ethylene glycol solution at 77 °K. This spectrum (Figure 1) presents vibronic structure with a characteristic quinone carbonyl group stretching frequency of 1600 cm^{-1}. Coupling of carbonyl stretching modes to the electronic state are typical of ketones with lowest nπ^* excited states. Electron-donating substituents on anthraquinones often introduce a charge-transfer excited state of lower energy

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(8) The acronym 2AQC2(HEt_2) fully describes the molecular structure as follows: 2 refers to the position of substitution on the AQ skeleton; AQ indicates that the molecule is a derivative of anthraquinone; C indicates that the substituent is linked to the ring system by a carboxamide group; 2 refers to the number of methylene groups in the chain; and (HEt_2) indicates that the terminal ammonium group is diethyl substituted. Other derivatives bearing sulfonamide or N-amide linkages are designated with S or A, respectively, in place of C.

(9) We have recently completed NMR studies that further support DNA intercalation of single-chained monocationic anthraquinone derivatives (manuscript in preparation).

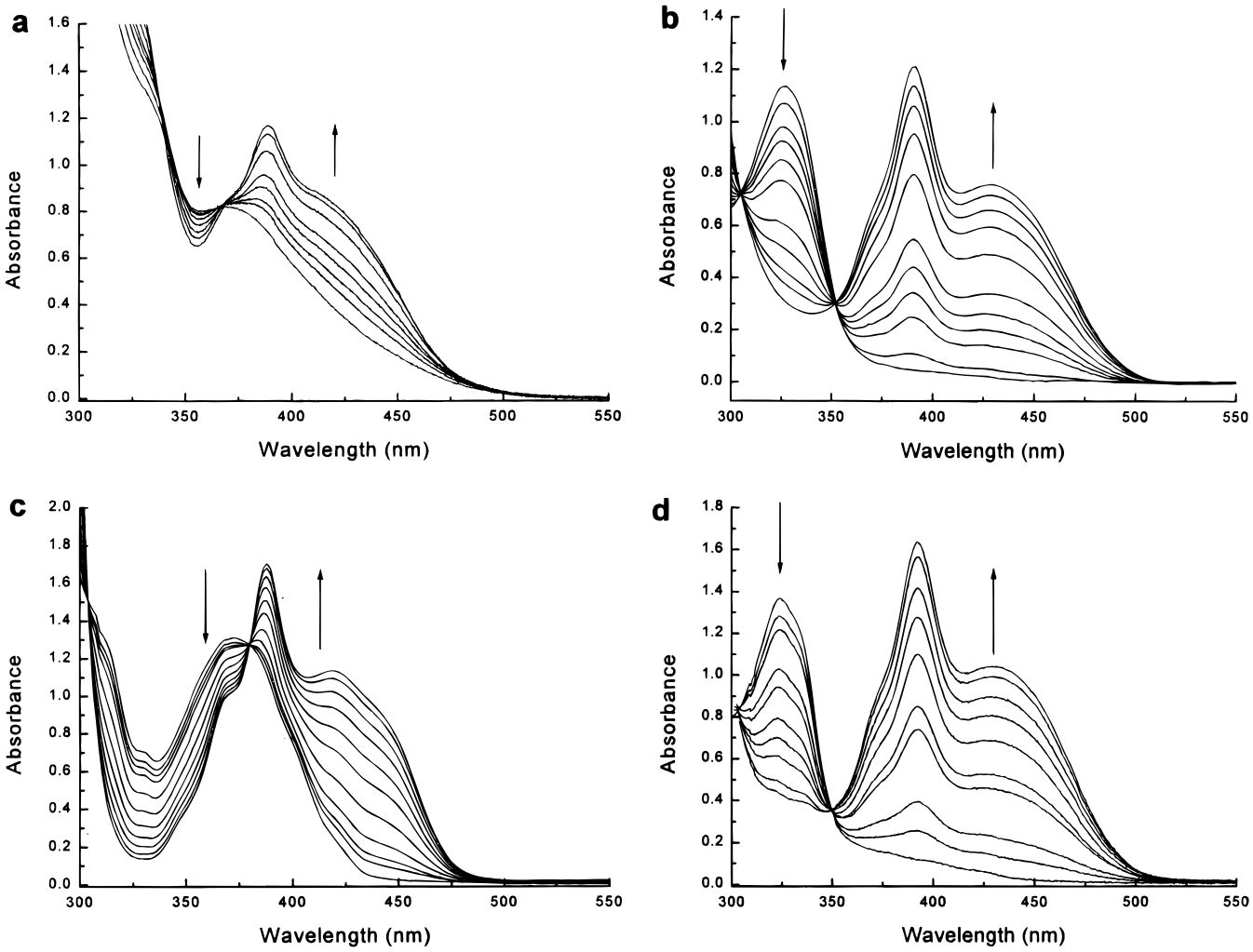
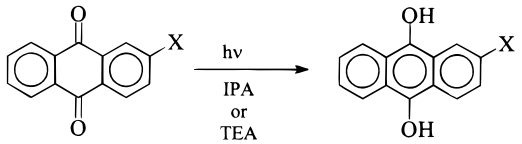


Figure 2. Photoreduction of the 0.2 mM anthraquinone derivatives by photolysis at 350 nm in isopropyl alcohol and triethylamine solutions monitored by UV−vis spectroscopy: (a) 2AQA2(HEt_2) in isopropyl alcohol at t = 0, 20, 40, 60, 80, 120, 160, and 180 min; (b) 2AQC2(HEt_2) in isopropyl alcohol at t = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 min; (c) 2AQA2(HEt_2) in triethylamine at t = 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 min; (d) 2AQC2(HEt_2) in triethylamine at t = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 min.

than the nπ* state of the parent compound.[10] Indeed, emissive properties similar to those observed for 2AQA2(HEt_2) have been attributed to charge-transfer states of substituted 2-aminoanthraquinones.[11,12]

(b) Photoreactions of 2AQA2(HEt_2) and 2AQC2(HEt_2) with Isopropyl Alcohol and Triethylamine. Anthraquinone derivatives having energetic nπ* excited states generally abstract a hydrogen atom from isopropyl alcohol to form ketyl radicals.[13] Further bimolecular reaction of these radicals often leads to irreversible photoreduction of the ketone. Corresponding carbonyl compounds having $\pi\pi$* or charge-transfer excited states react slowly, if at all, with isopropyl alcohol.[14] In contrast to the photoreduction of excited carbonyl compounds by direct hydrogen atom abstraction, the efficiencies of photoreduction by electron transfer from triethylamine are generally independent of the electronic configuration of the excited state.[15]

The photoreduction of 2AQA2(HEt_2) and 2AQC2(HEt_2) in neat, N_2-purged isopropyl alcohol was monitored by UV−vis spectroscopy (Figure 2). The two quinones were irradiated under identical conditions in a Rayonet reactor. Absorption spectra recorded as the reactions progressed exhibit clean isosbestic points, demonstrating the direct and quantitative conversion of the anthraquinone to a single photoproduct which was identified by comparison with authentic material as the corresponding hydroquinone (eq 1).



(1)

The relative rates of photoreduction of 2AQA2(HEt_2) and 2AQC2(HEt_2) with isopropyl alcohol were assessed by comparing the quantum yields for formation of the hydroquinones at low conversion. This analysis reveals that 2AQC2(HEt_2) reats with the alcohol more than 1 order of magnitude faster than 2AQA2(HEt_2) (Figure 3). In contrast, in triethylamine solvent, the photoreduction rates of 2AQA2(Et_2) and AQC2(HEt_2) are nearly identical. Further, the relative efficiency for photoreduction of 2AQA2(HEt_2) and 2AQC2(HEt_2) by triethylamine is not affected when the reaction is carried out in phosphate-buffered aqueous solution. However, the quantum yield ratio ($\Phi_{2AQA2(HEt_2)}/\Phi_{2AQC2(HEt_2)}$) for photoreduction by isopropyl

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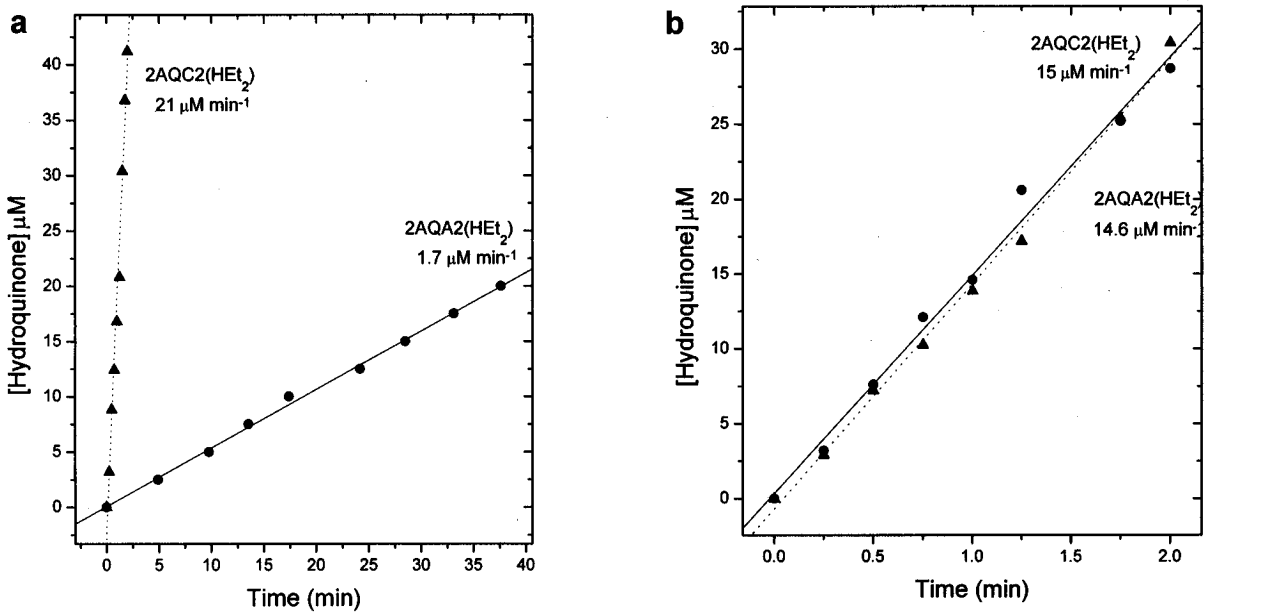

Figure 3. Rates of photoreduction of 2AQA2(HEt_2) and 2AQC2(HEt_2) in (a) isopropyl alcohol and (b) triethylamine solutions. The corresponding hydroquinone concentrations of 2AQA2(HEt_2) and 2AQC2(HEt_2) were monitored at 430 and 390 nm, respectively.

alcohol is reduced further to <0.01 with this change in solvent. Similar behavior reported for the photoreduction of aminobenzophenones[16] was attributed to a solvent effect on the relative contributions of nπ^* and charge-transfer states. The reduced reactivity of 2AQC2(HEt_2) in the aqueous solution is similarly ascribed to greater participation by a charge-transfer state due to solvent stabilization.

From the photochemical evidence, it is clear that 2AQA2(HEt_2) is a poorer hydrogen atom abstractor than 2AQC2(HEt_2). Conversely, both anthraquinone derivatives react with triethylamine by electron transfer with approximately equal efficiency. On this basis, it also seems likely that the lowest excited state of 2AQC2(HEt_2) has predominant nπ^* character and that of 2AQA2(HEt_2) has mostly $\pi\pi^*$ or charge-transfer character.

(2) Ground State Association Complexes of 2AQA2(HEt_2) and 2AQC2(HEt_2) with DNA. An important question to address is the nature of the associative interaction between the ground state of 2AQC2(HEt_2) and 2AQA2(HEt_2) with DNA. To examine this issue, equilibrium binding constants of the complexes were determined. In addition, the structure of the AQ−DNA complex was analyzed by the electronic absorption and induced circular dichroism spectroscopy.

The equilibrium binding constants of 2AQA2(HEt_2) and 2AQC2(HEt_2) with calf thymus DNA in 10 mM phosphate buffer solution were determined by an equilibrium dialysis method. The concentration of free quinone was determined directly or indirectly by fluorescence intensity measurements in 10 mM phosphate solution. Scatchard analysis of these data using the McGhee−Von Hippel equation reveals that 2AQA2(HEt_2) and 2AQC2(HEt_2) have similar binding constants, $K_a = 9.1 \times 10^5$ and 15.7×10^5 M^{-1} in 10 mM phosphate buffer, respectively.

The absorption spectra of both 2AQA2(HEt_2) and 2AQC2(HEt_2) are changed when they are complexed with CT-DNA. For instance, 2AQC2(HEt_2) undergoes a 3 nm bathochromic and shows a 23% hypochromicity upon complexation when compared with its spectrum in PBS solution. Similarly, 2AQA2(HEt_2) exhibits a slightly larger red shift (6 nm) and a 25% hypochromicity under the same conditions. The induced CD spectra of 2AQA2(HEt_2) and 2AQC2(HEt_2) with CT-DNA are shown in Figure 4. The complex of either quinone with the DNA induces a negative CD band in the region where only the anthraquinone absorbs. The intensity of the 2AQA2(HEt_2) band is approximately twice that of 2AQC2(HEt_2).

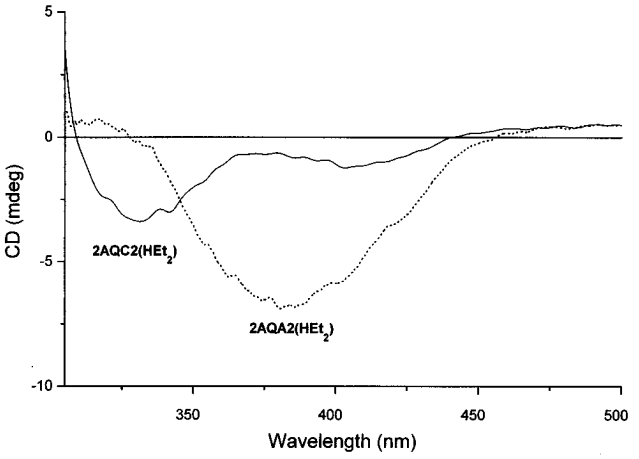

Figure 4. Induced CD spectra of 20 μM 2AQA2(HEt_2) and 2AQC2(HEt_2) complexed with 200 μM calf thymus DNA in PBS solution.

Table 1. Binding of 2AQC4(H_3) to Synthetic DNA Duplexes

5′-TATAGGTATATGGATAT-3′	5′-TATAGCTATATCGATAT-3′
3′-ATATCCATATACCTATA-5′	3′-ATATCGATATACGTATA-5′
oligo-I: $K_{assoc} = 1.4 \times 10^5$ M^{-1}	Oligo-II: $K_{assoc} = 1.6 \times 10^5$ M^{-1}

As mentioned above, GG-selective cleavage has been observed for these anthraquinone derivatives. At low quinone concentration this might be due to selective binding at GG sites. This possibility was examined by measuring the binding constants for 2AQC4(H_3) (see Chart 1 for structure) with the two oligonucleotides shown in Table 1. Oligo-I contains two GG steps but is otherwise the same as Oligo-II, which contains none. The association constants in PBS buffer show that there is no overwhelming preference for binding at GG steps.

(3) Emission and Time-Resolved Absorption Spectroscopy of 2AQA2(HEt_2), 2AQC2(HEt_2), and 2AQC4(H_3) with DNA. In order to understand the interaction of the excited states of DNA-intercalated 2AQA2(HEt_2) and 2AQC2(HEt_2) with CT-DNA, we examined the steady state emission and the transient

(16) Cohen, S. G.; Cohen, J. I. *J. Phys. Chem.* **1968**, *72*, 3872−93.

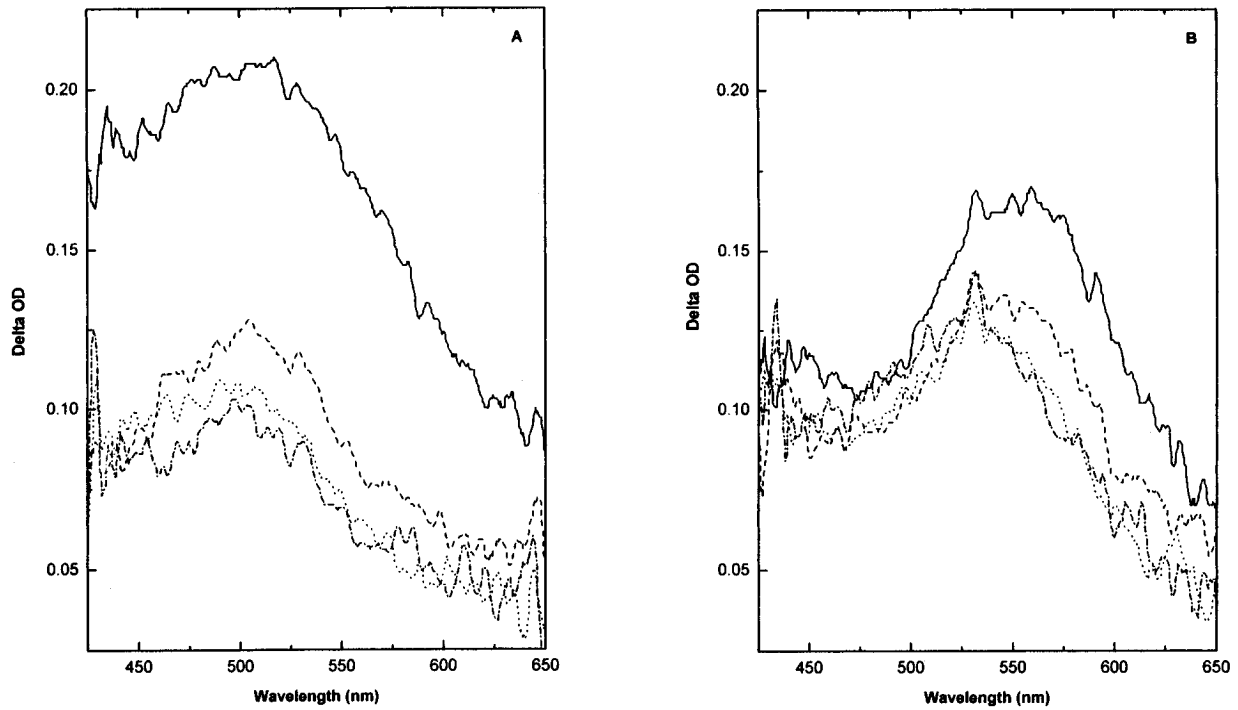

Figure 5. Transient absorbance spectra obtained from the flash photolysis of (a) 2AQA2(HEt_2) and (b) 2AQC4(HEt_2) (1.5 mM) complexed with 7.5 mM calf thymus DNA in air-saturated PBS solution. Spectra were recorded at 0 (solid), 50 (dashed), 100 (dotted), and 2000 (dot-dash) ps delays after the excitation pulse.

absorption spectra of the intermediates formed upon pulsed irradiation of the intercalated quinones.

The fluorescence of 2AQA2(HEt_2) is almost completely quenched by addition of a 10-fold excess of CT-DNA. Under the conditions of this experiment, the residual fluorescence observed from 2AQA2(HEt_2) probably results from the 4% of the quinone that is not bound at this DNA concentration (0.2 mM). Dynamic quenching of the quinone fluorescence by the DNA is negligible under these conditions because of the short lifetime of the singlet state of 2AQA2(HEt_2) and the low concentration of DNA. Similarly, 90% of the phosphorescence intensity of 2AQC2(HEt_2) in a 75% PBS−25% ethylene glycol glass is quenched by CT-DNA. In this experiment, approximately 82% of 2AQC2(HEt_2) is bound to DNA based on solution binding data in the absence of ethylene glycol.

Time-resolved laser flash photolysis experiments (355 nm, 20 ps) were carried out to help characterize the reactions initiated by irradiation of the anthraquinones when they are intercalated in DNA. Because of the higher concentrations required for these experiments and the relatively low aqueous solubility of 2AQC2(HEt_2), its more soluble analog 2AQC4(H_3) was used. This change is not expected to affect the functional properties of the excited state quinone.

Pulsed irradiation of a solution containing 2AQA2(HEt_2) or 2AQC4(H_3) and CT-DNA leads to the instantaneous (less than 20 ps) generation of transient species (Figure 5). These species were identified as the corresponding radical atoms of the anthraquinones by their independent generation with potassium superoxide in DMF. The radical anions formed in DMF are stable for several minutes, and their absorption spectra are shown in Figure 6. The spectra of the radical anions produced with superoxide are not identical with the DNA-bound transients. We ascribe these spectral shifts to solvent-induced effects that

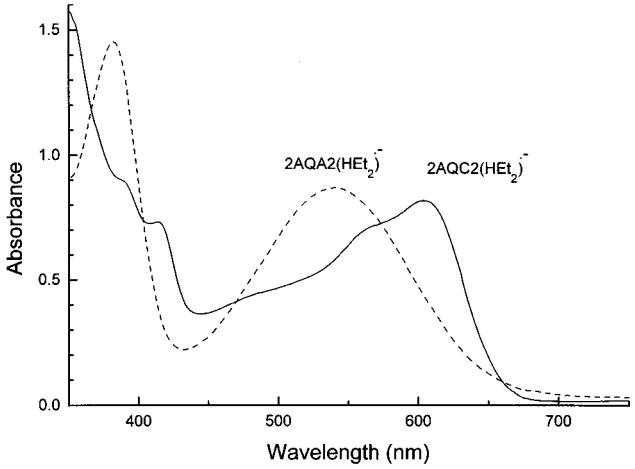

Figure 6. Absorption spectra of 2AQA2(HEt_2)$^{\bullet-}$ and 2AQA(HEt_2)$^{\bullet-}$ (0.2 mM) in DMF (1 cm pathlength).

are commonly observed for quinone radical anions.[17] Since the extinction coefficients of the 2AQC4(H_3) and 2AQA2(HEt_2) radical anions in DMS solution are nearly identical, the relative yield of DNA-bound quinone radical anions formed can be estimated by comparing their absorbancies in the laser flash photolysis experiment. On this basis, it appears that irradiations of DNA-bound 2AQA2(HEt_2) and 2AQC4(H_3) give nearly identical yields of their respective radical anions. In both cases, as expected in the heterogeneous environment of DNA, the transient radical anions decay following complex kinetic processes.

(4) DNA Cleavage Induced by Irradiation of 2AQA2-(HEt_2) and 2AQC2(HEt_2). The selectivity and efficiency of

(17) The absorption maximum of the anthraquinone radical anion is difficult to predict since it depends on solvent polarity and hydrogen bonding. Hamanoue, K.; Yokohama, K.; Kajiwara, Y.; Nakayama, T.; Teranishi, H. *Chem. Phys. Lett.* **1985**, *113*, 207−212. Hamanoue, K.; Nakayama, T.; Suguira, K.; Teranishi, H.; Washio, M.; Tagawa, S.; Tabata, Y. *Chem. Phys. Lett.* **1985**, *118*, 503−506.

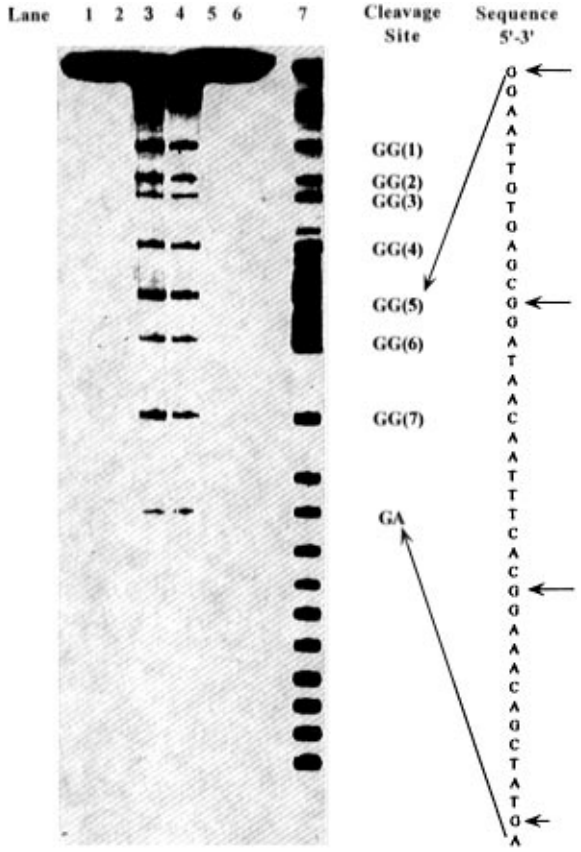

Figure 7. Autoradiogram comparing the photocleavage of a 3′ ^{32}P labeled 248 base pair restriction fragment by intercalated anthraquinones: lane 1, uncomplexed DNA dark control; lane 2, uncomplexed DNA irradiated; lane 3, irradiated DNA with 10 μM 2AQC2(HEt$_2$) after piperdine treatment; lane 4, irradiated DNA with 10 μM 2AQA2-(HEt$_2$) after piperdine treatment; lane 5, lane 3 before piperdine treatment; lane 6, lane 4 before piperdine treatment; lane 7, Maxam Gilbert G sequencing lane.

the DNA cleavage by 2AQA2(HEt$_2$) and 2AQC2(HEt$_2$) was examined by polyacrylamide gel electrophoresis of a radiolabeled restriction fragment consisting of 248 base pairs generated by *Eco*RI/*Pvu*II digestion of pA8G2 plasmid. The samples were irradiated in a Rayonet photoreactor equipped with 350 nm lamps. After irradiation was complete, the samples were divided. One aliquot received no treatment and was analyzed for spontaneous DNA backbone cleavage. The other portion was treated with hot piperidine to visualize alkaline labile damage. The autoradiograms recorded after photolysis of the DNA−anthraquinone mixtures are shown in Figure 7.

Close inspection of Figure 7 reveals that irradiation of either 2AQA2(HEt$_2$) or 2AQC2(HEt$_2$) causes DNA cleavage only after treatment with piperdine. Alkaline labile cleavage is typical of oxidative damage to a nucleic acid base.[18] Cleavage is usually spontaneous when it is initiated by hydrogen atom abstraction from the deoxyribose backbone.[19] Further, it is clear from analysis of Figure 7 that both 2AQA2(HEt$_2$) and 2AQC2-(HEt$_2$) cause cleavage primarily at 5′-G of GG steps in the DNA sequence.[20] Similar DNA cleavage patterns have been reported recently for riboflavin and other imides that were attributed to an electron-transfer reaction that yields a GG radical cation.[6] Molecular orbital calculations suggest that the GG radical cation is a thermodynamic sink for an electron hole in duplex DNA.[6a]

Dioxygen is required to generate GG-specific DNA cleavage by these irradiated quinones. Piperdine-treatable DNA cleavage is completely inhibited upon nitrogen purging before photolysis. Studies of other light-induced DNA cleaving agents have identified singlet oxygen ($^1O_2$),[21] formed by energy-transfer sensitization, or superoxide ($O_2^{\bullet-}$), generated by electron transfer,[22] as participants in damaging DNA. We investigated their possible role in the 2AQC2(HEt$_2$)-induced GG-selective photocleavage of DNA. The lifetime of ($^1O_2$) increases ca. 10-fold when the solvent is changed from H_2O to D_2O,[23] and this typically leads to an increased DNA cleavage efficiency in cases where $^1O_2$ participates.[24] We found that this solvent has no effect on the amount of DNA cleavage initiated by irradiation of the 2AQC2(HEt$_2$) complex. Thus, $^1O_2$ appears to play no, or at most a minor, part in DNA strand scission. Similarly, if reactions of $O_2^{\bullet-}$ initiated the DNA cleavage, this process would be inhibited by the enzyme superoxide dismutase (SOD). We observe no change in the efficiency of 2AQC2(HEt$_2$)-induced DNA cleavage when SOD is present. On this basis we conclude that $O_2^{\bullet-}$ plays little or no role in this cleavage reaction.[25]

Although nearly all (>96%) of the 2AQA2(HEt$_2$) and 2AQC2(HEt$_2$) is bound to DNA under the conditions of the cleavage experiment, it is unclear whether the observed GG selectivity originates from free or bound quinone. Since both 2AQA2(HEt$_2$) and 2AQC2(HEt$_2$) intercalate in double-stranded DNA, increasing the concentration of the quinone to a value greater than the number of intercalation sites will ensure that a significant fraction of the quinone is free in solution.

When the concentration of 2AQC2(HEt$_2$) is 10 μM and the DNA is 50 μM in base pairs, calculation from the binding constant indicates that every fifth intercalation site is occupiedand that the concentration of free quinone is less than 0.5 μM. Photolysis of the complex at this concentration yields the usual pattern of GG-selective cleavage after treatment with piperidine when analyzed by gel electrophoresis (lane 4, Figure 7). When the concentration of 2AQC2(HEt$_2$) is increased to 50 μM, in principle, every intercalation site in the DNA will be occupied. Under these conditions, with identical absorbed light doses, the piperdine-induced cleavage efficiency is unchanged and the pattern of GG-selective cleavage is maintained (data not shown). When the concentration of 2AQC2(HEt$_2$) is increased further to 100 μM, there are considerably more quinone molecules than there are intercalation binding sites and the concentration of nonintercalated 2AQC2(HEt$_2$) must be at least 75 μM. At this high AQ−DNA ratio (lane 2, Figure 8), there is a significant amount of spontaneous nonselective cleavage of the DNA. On this basis, it appears that the piperidine-requiring GG-selective cleavage of 2AQA2(HEt$_2$) and 2AQC2(HEt$_2$) is initiated by irradiation of quinone bound to the DNA by intercalation. Since even at the lowest quinone concentration examined intercalation must occur at sites other than GG, these findings, too, indicate that the GG-selective

(18) Modified bases like 8-oxoguanine are released from duplex DNA only after piperdine treatment. Tchou, J.; Kasia, H.; Shibutani, S.; Chung, M.-H.; Laval, J.; Grollman, A. P.; Hishimura, S. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4690−4694.

(19) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107−1136.

(20) There are also some weaker signals in Figure 7 indicating cleavage at the G of some 5′-GA-3′ steps.

(21) While $^1O_2$ damage has been recorded in many instances, it has recently been described for an anthraquinone derivative: Reszka, J. K.; Bilski, P.; Chignell, C. F.; Hartley, J. A.; Khan, N.; Souhami, R. L.; Mendonca, A. J.; Lown, J. W. *J. Photochem. Photobiol. B: Biol.* **1992**, *15*, 317−35.

(22) Hertzberg, R. P.; Dervan, P. B. *Biochemistry* **1984**, *23*, 3934−3945.

(23) Merkel, P. B.; Kearns, D. R. *J. Am. Chem.* **1972**, *94*, 1029−1030.

(24) The D_2O-H_2O effect has been clearly demonstrated in the methylene blue sensitized DNA cleavage. Schneider, J. E., Jr.; Phillips, J. R.; Pye, Q.; Maidt, M. L.; Price, S.; Floyd, R. A. *Arch. Biochem. Biophys.* **1993**, *301*, 91−97. Floyd, R. A.; West, M. S.; Eneff, K. L.; Schneider, J. E. *Arch. Biochem. Biophys.* **1989**, *273*, 106−111.

(25) We have previously observed that $^1O_2$ and O_2^- are not involved in DNA cleavage with other anthraquinone derivatives (ref 5).

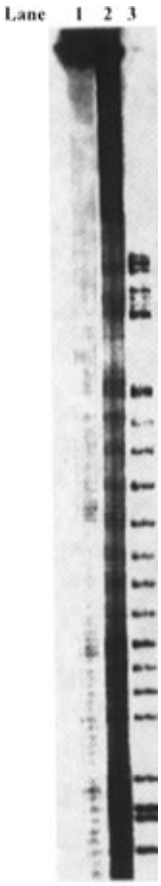

Figure 8. Autoradiogram comparing the photocleavage of a 3′ ^{32}P labeled 248 base pair restriction fragment by nonintercalated anthraquinones (total DNA concentration 50 μM base pairs): lane 1, irradiated with 100 μM 2AQA2(HEt_2) added; lane 2, 100 μM 2AQC2(HEt_2) irradiated with 2AQC2(HEt_2) added; lane 3, Maxam Gilbert G sequencing lane.

cleavage cannot be due to GG-selective binding. The nonselective spontaneous cleavage observed at high 2AQC2(HEt_2) concentrations must originate with nonintercalated quinone. Significantly, irradiation of high concentrations of 2AQA2(HEt_2), where the concentration of nonintercalated quinone must also be at least 75 μM, does not give any nonselective spontaneous cleavage (lane 1, Figure 8).

Discussion

(1) Photochemistry and Photophysics of 2AQA2(HEt_2) and 2AQC2(HEt_2) in Solution and with DNA. The anthraquinone photonucleases are built from three pieces: (1) the anthraquinone chromophore, (2) a linker group, and (3) a cationic ammonium side chain. The function of the chromophore is to absorb light and initiate chemical transformations of the DNA. The cationic side chain serves two purposes: to increase the water solubility of the compound and to provide increased binding to DNA by electrostatic attraction. The linker group provides, at least, a convenient means of tethering the chromophore and ammonium group.

The carboxamide group of 2AQC2(HEt_2) not only links the ammonium terminus with the anthraquinone ring but also modifies the properties of the excited state quinone. As a relatively electron deficient anthraquinone derivative, 2AQC2(HEt_2) is expected to behave like typical reactive nπ^* carbonyl compounds.[26] In contrast, as a derivative of 2-aminoanthraquinone, the linking group of 2AQA2(HEt_2) is electron releasing and is expected to alter the nature of the lowest excited state of the quinone so that it has predominant $\pi\pi^*$ or charge-transfer character.[27] The spectroscopic and chemical evidence described above clearly supports this view. Thus, reversing the structure of the linking group has altered the electronic configuration of the reacting excited state. Of particular relevance to this work are the experiments that demonstrate that excited 2AQC2(HEt_2) is reduced effectively by hydrogen atom abstraction from isopropyl alcohol and by electron transfer from triethylamine, but that excited 2AQA2(HEt_2) reacts only by electron transfer from triethylamine. This difference in chemical properties provides a solid basis for analyzing whether hydrogen atom abstraction from deoxyribose or electron transfer from a DNA base is the reaction that initiates GG strand cleavage.

Thermodynamic considerations play a key role in assessing the likelihood that excited 2AQA2(HEt_2) and 2AQC2(HEt_2) will react with DNA by electron transfer. The oxidation potentials of the nucleic acid bases have been determined.[28] Guanine has the lowest oxidation potential and, as expected, the purines are more easily oxidized than are the pyrimidines. The reduction potentials of 2AQA2(HEt_2) and 2AQC2(HEt_2) are -0.68 and -0.58 V (vs SCE), respectively. Substitution of these values into the Weller equation[29] (eq 2) reveals that electron transfer from any of the DNA bases to the singlet or triplet excited state of 2AQA2(HEt_2) or 2AQC2(HEt_2) is highly exothermic and therefore feasible on thermodynamic grounds and likely to be rapid.

$$\Delta G_{et} = -(E_{D/D-} - E_{A/A+} + \Delta E_{0,0}) \qquad (2)$$

The spectroscopic data indicate rapid reaction of the excited anthraquinones with DNA. The emissions of both 2AQA2(HEt_2) and 2AQC2(HEt_2) are quenched when they are complexed with DNA. Laser flash photolysis of 2AQA2(HEt_2) or 2AQC2(HEt_2) bound to DNA leads to the formation of their radical anions in less than 20 ps, confirming the thermodynamic prediction that electron transfer will be spontaneous. The specific DNA base that serves as the source of the electron cannot be identified from this experiment, but critically, the excited state electron transfer pathway for both quinones is firmly established.

(2) Ground State Quinone−DNA Complexes. In order to assess the nuclease activities of 2AQA2(HEt_2) and 2AQC2(HEt_2), the nature of their association with DNA must be understood. The equilibrium binding constants with CT-DNA of the two quinones are nearly identical, suggesting that the precise structure of the linking group does not affect the thermodynamics of association. The spectral properties of the bound quinones provide some additional structural details.

X-ray crystal structures of anthracycline drugs, which contain an anthraquinone subunit, reveal that they bind to DNA by intercalation.[30] The bathochromic and hypochromic shifts of the absorptions of bound 2AQA2(HEt_2) and 2AQC2(HEt_2) as well as their induced CD spectra indicate that they are also intercalated. The induced CD spectra of 2AQA2(HEt_2) and 2AQC2(HEt_2) have identical shapes but different intensities.

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(27) Allen, N. S.; McKellar, J. F. *J. Photochem.* **1976**, *7*, 107−111.

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(29) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259−263. The exergonicity of electron transfer between the DNA bases and the excited quinones can be calculated from the base oxidation potentials in ref 26, the quinone redox potentials, and the 0,0 energies we report, ΔG_{et} (2AQA2(HEt_2)) = -1.35, -1.23, -1.17, and -1.14 V for G, A, C, and T, respectively.

(30) Quigley, G. J.; Wang, A. H.-J.; Ughetto, G.; van der Marcel, G.; van Boom, J. H.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7204−7208.

Since the relative intensities of these bands do not reflect differences in binding affinity, this difference may indicate a subtle change in intercalation site or a shift of the chromophore relative to the center of the intercalation site.[31] These differences in orientation are unlikely to affect the rate of electron transfer since the quinones are in contact with the bases and π-electron overlap should not depend strongly on relative orientation. In contrast, a small change in the structure of the intercalated quinone may significantly influence the efficiency of hydrogen atom abstraction since this process is strongly dependent on the distance and orientation of the carbonyl oxygen atom and the hydrogen atom.[32]

(3) Selectivity and Efficiency of DNA Cleavage by 2AQA2-(HEt_2) and 2AQC2(HEt_2). Comparison of the cleavage patterns and efficiencies induced by irradiation of 2AQA2(HEt_2) and 2AQC2(HEt_2) bound to the restriction fragment in combination with the spectroscopic and chemical analysis permits assignment of a reaction mechanism. When the ratio of the quinone to DNA base pairs is 1:5, both 2AQA2(HEt_2) and 2AQC2(HEt_2) cleave DNA with equal efficiency and show the same GG selectivity. Since the chemical and spectroscopic results indicate that only 2AQC2(HEt_2) can abstract a hydrogen atom, we conclude that the piperidine-activated GG-selective cleavage is initiated by electron transfer to form a base radical cation.

The experiments at high quinone concentration reveal a different pattern of reactivity and selectivity. At high concentration of the quinone, where some is not intercalated, irradiation of 2AQC2(HEt_2) gives spontaneous, nonselective cleavage, but cleavage by 2AQA2(HEt_2) remains GG-selective and still requires piperidine activation. These findings indicate that the GG-selective cleavage is initiated by either of the quinones when intercalated, but only nonintercalated 2AQC2(HEt_2) gives spontaneous nonselective cleavage. This result is consistent with the chemical and spectroscopic properties of the quinones which indicate that nonintercalated excited 2AQC2(HEt_2) is capable of direct hydrogen atom abstraction from a deoxyribose unit, a process that 2AQA2(HEt_2) cannot undergo.

(4) GG-Selectivity by Hole Hopping. The equilibrium association constant experiments and the observation that GG-selective cleavage occurs even when essentially all intercalation sites are occupied rules out selective binding as the cause of the GG-selective cleavage. Electron transfer to an excited intercalated quinone generates a base radical cation and the quinone radical anion. Reaction of the base radical cation to produce strand cleavage must compete with back electron transfer, which is expected to be rapid when the radical cation and radical anion are in direct contact. The time-resolved laser spectroscopy reveals multiple decay rates for the quinone radical anion. Some radical anions decay within a few picoseconds of the laser pulse, others live more than 10 ns. The long-lived radical anions cannot be in direct contact with a base radical cation. Either the first-formed base radical cation has been consumed by a chemical reaction or the radical cation has migrated away from the intercalation site containing the radical anion.

An attractive explanation for the GG-selective cleavage is radical cation migration in the oxidized DNA. Recent calculations indicate that GG steps have particularly low oxidation potentials.[6] The oxygen-dependent cleavage efficiency suggests that the guanyl radical cation itself or a subsequently formed chemical intermediate may be trapped by O_2. Furthermore, Kasai, Cadet, and co-workers[33] have recently demonstrated that guanine radical cations react with water, leading to damage that is revealed by hot piperidine treatment.[18] Migration of a base radical cation away from the intercalation site of its generation to a GG trap can proceed downhill thermodynamically for some base pair sequences.[34] This separation in space will slow the rate of back electron transfer and provide time for GG radical cations to react with water or O_2, accounting for the observed GG selectivity. Experiments are underway to test this hypothesis.

Conclusions

2AQC2(HEt_2) has lowest energy nπ* excited states and is capable of reaction by electron transfer or hydrogen atom abstraction. 2AQA2(HEt_2) has lowest energy charge-transfer or $\pi\pi$* states and can react only by electron transfer. Electron transfer from either intercalated quinone forms a base radical cation and leads, eventually, to GG-selective strand cleavage. Radical cation migration through double-stranded DNA may account for the GG-selectivity. Direct hydrogen atom abstraction by a nonintercalated excited 2AQC2(HEt_2) leads to spontaneous, nonselective cleavage of DNA.

Experimental Section

Materials. The syntheses of anthraquinone-2-carbonyl chloride and 2AQC4(H_3) have already been reported,[4] and the material obtained by these procedures gave satisfactory NMR spectra and melting points. Spectrograde ethylene glycol (Eastman Kodak) was used in the low-temperature phosphorescence studies. The phosphate buffer used, unless specified otherwise, is 10 mM phosphate with 100 mM NaCl at pH = 7.2 (denoted as PBS). Calf thymus DNA and superoxide dismutase from bovine erthyocytes were purchased from Sigma Co. PA8G2 plasmid was kindly supplied by Dr. Peter Nielsen of the University of Copenhagen. Restriction enzymes *Eco*RI and *Pvu*II and DNA polymerase (Klenow fragment) were purchased from New England Biolabs. [α-^{32}P]dATP was purchased from Amersham. *N*,*N*-Diethylenediamine, 2-aminoanthraquinone, 3-bromopropionyl chloride, diethylamine, isopropyl alcohol, triethylamine, dimethylformamide, D_2O (99%), and potassium superoxide were purchased from Aldrich Chemical Co. In all cases the chemicals were used without further purification. Cellulose dialysis tubing (2000 MW cutoff) was manufactured by Spectrum Medical and was washed several times with water prior to use.

Instrumentation. NMR spectra were recorded on a Varian Gemini-300 spectrometer. CD spectra of the DNA−ligand complexes were recorded on a Jasco J-270 instrument. Fluorescence and phosphorescence spectra were measured using a Spex-1681 Fluorlog 2. Phosphorescence spectra were measured in a 25% ethylene glycol−aqueous phosphate buffer solvent cooled to a 77 °K glassy matrix. A Cary 1E spectrometer was used to record UV−vis spectra. A Bioanalytical Systems 100 potentiostat was used to record cyclic voltammetry traces. Excited state lifetime measurements were performed using a Photon Technology LS-1 time-resolved fluorimeter. Steady state irradiations were conducted in a Rayonet RPR-100 photoreactor. The picosecond transient spectroscopy laser apparatus has been described elsewhere.[35]

***N*-[[2-(*N*′,*N*′-Diethylamino)ethyl]amino]-2-anthraquinonecarboxamide Hydrochloride (2AQC2(HEt_2))** was prepared by suspending 0.25 g of anthraquinone-2-carbonyl chloride in 20 mL of methylene chloride and adding a solution of 0.11 g of *N*,*N*-(diethylamino)-ethylamine in 10 mL of methylene chloride. The solution was stirred for 2 h after the addition was complete. The product was isolated by precipitation of the reaction mixture in 200 mL of diethyl ether.

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(33) Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1992**, *114*, 9692−9694.

(34) For example, if the quinone [AQ] is intercalated at the 5′ end of the sequence 5′-[AQ]AGG-3′, the first-formed $A^{\bullet+}$ will migrate to GG, leaving neutral A between the radical cation and radical anion.

(35) Zhu, Y.; Koefod, R. S.; Devadoss, C.; Shapley, J. R.; Schuster *Inorg. Chem.* **1992**, *31*, 3505−3506.

Recrystallization from isopropyl alcohol gave 0.28 g (76% yield) of light beige crystals: mp 181−182 °C; ^{1}H NMR (DMSOd_6) δ 1.23 (t, 6 H, J = 7.3 Hz), 3.17 (q, 4 H, J = 7.3 Hz), 3.24 (t, 2 H, J = 6.5 Hz), 3.69 (q, 2 H, J = 6.5, 5.6 Hz), 7.94 (d, 1 H, J = 3.3 Hz), 7.96 (d, 1 H, J = 3.3 Hz), 8.22 (t, 1 H, J = 5.6 Hz), 8.23 (t, 1H, J = 5.6 Hz), 8.28 (d, 1 H, J = 8.1 Hz), 8.40 (dd, 1 H, J = 1.7, 8.0 Hz), 8.66 (d, 1 H, J = 1.7 Hz), 9.32 (t, 1 H, J = 5.6 Hz); ^{13}C NMR (DMSO-d_6) δ 8.37, 34.53, 46.43, 49.58, 125.97, 127.11, 127.29, 127.38, 127.59, 133.17, 133.27, 133.30, 133.37, 134.73, 135.03, 139.02, 165.52, 182.45, 182.55; HRMS 351.1709 (calcd), 351.1709 (found).

2-(3-Bromopropionamido)anthraquinone was prepared by refluxing 1.0 g of 2-aminoanthraquinone in 5 mL of freshly distilled 3-bromopropionyl chloride for 2 h. After cooling, the product was collected by filtration and washed with diethyl ether. Recrystallization from ethanol gave 1.5 g (94% yield) of yellow crystals: mp 254 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.05 (t, 2 H, J = 6.3 Hz), 3.76 (t, 2 H, J = 6.3 Hz), 7.91 (d, 1 H, J = 4.2 Hz), 7.93 (d, 1 H, J = 2.2 Hz), 8.00 (dd, 1 H, J = 2.2, 6.4 Hz), 8.14−8.20 (m, 3 H), 8.48 (d, 1 H, J = 2.2 Hz), 10.79 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 28.71, 39.12, 116.14, 124.09, 127.02, 127.10, 128.40, 128.93, 133.46, 133.48, 134.56, 134.63, 134.68, 134.97, 144.73, 169.78, 181.91, 182.98; HRMS 357.0001 (calcd), 356.9999 (found).

2-[3-(*N*,*N*-Diethylamino)acetamido]anthraquinone (2AQA2(HEt$_2$). A solution of 4 mL of diethylamine and 2-(3-bromopropionamido)-anthraquinone was heated at reflux for 1 h. The solvent was removed under reduced pressure, and the remaining solid was dissolved in a HCl-saturated ethanol solution and then precipitated with 300 mL of diethyl ether. Recrystallization from isopropyl alcohol gave 0.7 g (65% yield) of yellow crystals: mp 244−245 °C; ^{1}H NMR (DMSO-d_6) δ 1.24 (t, 6 H, J = 7.2 Hz), 2.97 (t, 2 H, J = 7.2 Hz), 3.15 (q, 4 H, J = 7.3 Hz), 3.39 (t, 2 H, J = 7.3 Hz), 7.89 (d, 1 H, J = 3.9 Hz), 7.92 (d, 1 H, J = 3.9 Hz), 8.07 (dd, 1 H, J = 2.1, 6.5 Hz), 8.16 (s, 1H), 8.17−8.20 (m, 2 H), 8.50 (d, 1 H, J = 2.1 Hz), 11.02 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 8.47, 30.81, 46.71, 46.73, 116.19, 124.15, 127.00, 127.07, 128.41, 128.86, 133.44, 133.47, 134.52, 134.63, 134.98, 144.70, 169.51, 181.89, 182.97; HRMS 351.1709 (calcd), 351.1717 (found).

Binding Constants. The affinities of the anthraquinone derivatives for calf thymus DNA were evaluated by an equilibrium dialysis method. A 100 μL aliquot of approximately 5 mM concentration in base pairs was placed and sealed in a 5 cm section of dialysis tubing. The dialysis bags were suspended in solutions containing the quinone at various concentrations. Each dialysis sample was stirred constantly at room temperature for 48 h. Preliminary experiments showed that a 24 h period was sufficient to reach equilibrium. The concentration of free 2AQA2(HEt$_2$) was determined directly by quantitative fluorescence measurements. 2AQC2(HEt$_2$) was reduced photochemically to the hydroquinone with isopropyl alcohol under N_2 prior to fluorescence analysis. The concentrations of quinone were determined from calibration curves. The binding isotherms were fit using the Scatchard analysis[36] and the Mc Ghee−Von Hippel equation.[37]

Chemical Reduction of the Anthraquinones. Hydroquinones were prepared by reduction with hydrosulfite. The semiquinone radical anions were prepared by treating 0.2 mM anthraquinone in a N_2-purged DMF solution (5 mL) with 10 mg of KO_2. After 5 min, the excess KO_2 was filtered away and the colored solution was transferred to a sealed cuvette.

DNA Cleavage. A 248 base pair *Eco*R1/*Pvu*II restriction fragment was prepared from pA8G2, a derivative of pUC19, containing the sequence 5′-GT$_4$CT$_2$CT$_2$CTGCA-3′•5′-GA$_2$GA$_2$GA$_4$CTGCA-3′ cloned into the *Pst*I site. The restriction fragment was radiolabeled at the 3′ end with [α-^{32}P]dATP using the Klenow fragment DNA polymerase.[38] Solutions (10 μL) prepared for analysis of photoinduced cleavage had the following concentrations of reagents: 10 mM sodium phosphate (pH = 7.0), 10 μM AQ, 50 μM calf thymus carrier DNA, 2500 cpm [^{32}P]DNA. The samples were irradiated for ca. 2 h at 350 nm in 1.5 mL plastic microcentrifuge tubes placed on a rotating carousel in a Rayonet reactor equipped with four bulbs. Control samples were subjected to the identical procedure but were wrapped in aluminum foil throughout the irradiation period. The reaction products were isolated by precipitation with cold ethanol. Inorganic salts were removed by washing with 80% ethanol. The samples were divided: some were treated with 1 M aqueous piperdine (30 min at 90 °C), and the others were analyzed directly. Electrophoresis of the photolysis mixture was conducted on an 8% denaturing polyacrylamide gel at 65 W constant power. DNA cleavage patterns on the radiograms were visualized after exposure of photographic film to the dried gel for 1−2 days. A lane containing the Maxam−Gilbert A+G or G sequencing reaction[39] was run simultaneously to determine the precise cleavage site.

Laser Flash Photolysis Studies of the Anthraquinone Derivative−DNA Complexes. A solution containing 1.5 mM of the appropriate anthraquinone and 7.5 mM sonicated self-thymus DNA in PBS was excited with a 20 ps duration pulse of 355 nm light from a Nd-YAG laser.[9] Transient absorbance spectra were recorded at delays of 0 ps, 50 ps, 100 os, and 2 ns after the excitation laser pulse.

Electrochemical Measurements. A three-electrode cell configuration, consisting of a platinum working electrode, a saturated calomel reference electrode, and a platinum auxiliary electrode, was used. The supporting electrolyte was 0.1 M aqueous sodium phosphate buffered at pH = 7 which had been N_2 purged to remove molecular oxygen.

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