

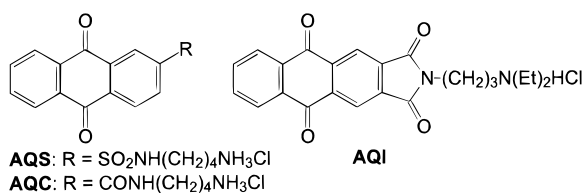
Anthraquinone Photocleavage Structure Determines Its Mode of Binding to DNA and the Cleavage Chemistry Observed

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The development of chemical agents that cleave the backbone of DNA has been spurred by a desire to isolate functional DNA sequences¹ and to develop agents that image (footprint) molecules bound to DNA.² Synthetic nucleases are structurally diverse compounds that operate by three general mechanisms: hydrolysis of the sugar phosphate bond,³ chemical modification of a DNA base,^{1a,b,4} or hydrogen abstraction from a deoxyribose unit.⁵ We recently described a set of anthraquinone derivatives that act as photocleavages.⁶



Three classes of nuclease behavior that depend on the structure of the quinone and the reaction conditions have been observed. When intercalated into duplex DNA, irradiation of quinones such as AQC or AQS in Cl⁻ free solution gives selective cleavage at the 5'-G of GG steps⁷ that is revealed only after treatment of the DNA with hot piperidine.⁸ In contrast, spontaneous (no piperidine treatment required), nonselective cleavage is observed when AQC is irradiated in a solution containing Cl⁻.^{6c} Finally, irradiation of DNA saturated with AQC, so that some quinone is free in solution, leads to nonsequence-selective spontaneous cleavage.^{6c} These findings indicate that the mechanism of reaction controls the characteristics of the DNA cleavage. Irradiation of an intercalated quinone initiates electron transfer to form a base radical cation

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(8) There is comparatively minor amounts of cleavage at the G of 5'-GA-3' steps.

and results in GG selective cleavage. Irradiation of unbound AQC results in hydrogen atom abstraction from a deoxyribose and causes spontaneous cleavage. We describe herein a new quinone nuclease with unique properties. AQI⁹ binds nonintercalatively to DNA, and its irradiation gives spontaneous, nonsequence-selective cleavage of DNA that can be used for photofootprinting of ligands bound in the minor groove.

We conducted a series of experiments to determine if AQI binds to DNA by intercalation or by association with a groove. A competitive ethidium bromide displacement method was used to evaluate the binding of AQI in PBS¹⁰ solution to DNA. The association constants for AQI with [poly(dA)/poly(dT)] and with duplex poly[(dGdC)] are 0.5 and $3.9 \times 10^5 \text{ M}^{-1}$, respectively. These values are similar to that measured for AQC with poly[(dGdC)],^{6b} for example.

One indicator of DNA binding mode is a change in viscosity when a small molecule associates with DNA. Intercalation increases the length of DNA and significantly increases the viscosity, whereas groove binding typically has a much smaller effect on viscosity.¹¹ We compared the effect of equivalent amounts of AQS and AQI on the viscosity of calf-thymus DNA solutions. The results reveal an increase in viscosity characteristic of intercalators for AQS. In contrast, AQI has essentially no effect on the viscosity, suggesting that it does not bind by intercalation.¹²

A second indicator of DNA binding mode is circular dichroism spectroscopy. DNA provides a chiral environment that will induce a CD spectrum in a bound ligand.¹³ The induced CD spectrum for AQI with calf-thymus DNA in PBS solution is biphasic and has more than twice the intensity of CD spectra observed for AQC or AQS. This result, too, supports groove binding for AQI since this mode typically induces a stronger CD spectrum than does intercalation.¹⁴

A third, and especially convincing, means to distinguish intercalative and groove binding to DNA is scanning force microscopy (SFM).¹⁵ Definitive evidence that AQS and AQI bind by different modes comes from visualization of individual DNA molecules by SFM. Images of the linearized plasmid, *pBluBacHis* (*pBBH*) without quinone yield an average length of 3430 nm ($n = 156$, $\sigma = 75$ nm). Images acquired following immobilization of the plasmid from an AQS solution (15 μM) reveal lengthening of the DNA by 300 nm.¹² In contrast, images acquired following immobilization from an AQI solution reveal no measurable change in DNA length. Figure 1 shows these effects quantitatively. Competition with ethidium bromide verifies that AQI blocks the minor groove.¹⁶

The change of binding mode changes the reactions of the excited state of these quinones with DNA. AQI is not

(9) For 2,3-anthraquinonedicarboxylic acid anhydride, see: Arient, J.; Podstata, J. *Collect. Czech. Chem. Commun.* **1974**, *39*, 3117–3123. Its reaction with (*N,N*-diethylamino)propylamine in refluxing acetic acid gave AQI: mp 258 °C (dec); ¹H NMR (DMSO-*d*₆) δ 1.04 (6H, t, $J = 7.2$ Hz), 1.96 (2H, q, $J = 7.2, 4.7$ Hz), 2.70–2.77 (6H, m), 3.73 (2, t, $J = 7.2$ Hz), 7.78 (2H, dd, $J = 3.5, 2.4$ Hz), 8.26 (2H, dd, $J = 3.5, 2.4$ Hz), 8.67 (2H, s); ¹³C NMR (D₂O) δ 188.32, 166.09, 138.20, 136.44, 135.47, 133.32, 127.47, 129.82, 56.89, 55.14, 42.66, 41.10 15.75; HRMS calcd 390.1620, found 390.1607.

(10) PBS is 100 mM NaCl, 10 mM sodium phosphate at pH = 7.

(11) Examples of intercalators include anthracyclines: (a) Reinert, K. E. *Nucleic Acids Res.* **1983**, *11*, 3411–3430. Groove binders: (b) Reinert K. E.; Stutter, E.; Schweiss, H. *Nucleic Acids Res.* **1979**, *7*, 1375–1392 (netropsin). (c) Reinert, K. E. *Biophys. Chem.* **1981**, *13*, 1–14 (distamycin).

(12) See Supporting Information.

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(16) Incubation of *pBBH* with 10 μM AQI followed by addition of 5 μM ethidium bromide does not lengthen the DNA. The expected lengthening due to 5 μM ethidium alone is ca. 360 nm based on $K = 6.6 \times 10^4 \text{ M}^{-1}$ and an exclusion number of 2 as previously determined by SFM assay.¹⁴

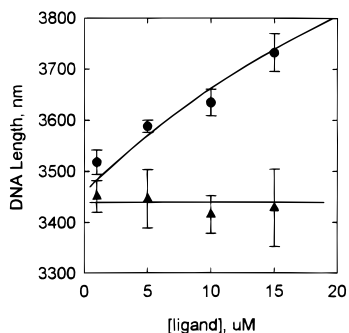


Figure 1. Length versus ligand concentration for AQS (▲) and AQI (●). The error bars represent 2σ . Eighty-eight high-resolution images of individual quinone-*p*BBH complexes were acquired and measured.²³

luminescent in aqueous solution at room temperature. However, a phosphorescence emission characteristic of an $n\pi^*$ excited state is detected from a frozen ethylene glycol-PBS solution at 77 K.¹⁷ When bound to [poly(dA)/poly(dT)] or duplex poly[(dGdC)], the phosphorescence of AQI is shifted 7 nm to higher energy and its intensity is reduced 24% and 62%, respectively.¹⁸ In contrast, the phosphorescence of AQS in frozen solution is completely quenched (>95%) when it is intercalated in DNA. Further, laser flash photolysis of AQI bound to calf-thymus DNA shows no formation of the radical anion out to 1 ns. Similar irradiation of bound AQC shows the strong absorption of the quinone radical anion in less than 20 ps.¹² The laser spectroscopy and the incomplete phosphorescence quenching for AQI show that the rate of electron transfer is slowed significantly when the quinone is not intercalated.

The change of binding mode also has a remarkable effect on the light-induced cleavage of duplex DNA by these quinones. A polyacrylamide gel autoradiogram of a ³²P-3'-end-labeled 248-base restriction fragment that was cleaved by irradiation of AQI¹⁹ shows spontaneous cleavage with low quantum efficiency²⁰ and essentially equal effectiveness at every nucleotide.¹² In contrast, irradiation of AQS or AQC under these conditions gives only insignificant amounts of spontaneous cleavage, and treatment of these samples with hot piperidine reveals selective cleavage at the 5'-G of GG steps. Treatment of the restriction fragment with piperidine after irradiation of AQI does not reveal any additional cleavage sites. Clearly, the change in binding mode alters the reactions of the quinone excited state and controls the character of the DNA cleavage.

The site of reaction for reagents that attack and cleave DNA can be assessed by employing ligands known to bind selectively

(17) Turro, N. J. *Modern Molecular Photochemistry*; Benjamin Cummings: Menlo Park, CA, 1975; Chapter 7.

(18) A small fraction of the differential quenching by the two DNA polymers may be due to differences in binding constant. We estimate under these conditions that >97% and 80% of AQI is bound to poly[(dGdC)] and [poly(dA)/poly(dT)], respectively.

(19) The restriction fragment is excised from pUC-19 plasmid using *EcoRI*-*PvuII* enzymes. The labeling procedure are found in ref 6d. From the binding constant, we estimate that >90% of the imide is bound during the photolysis.

(20) A comparison of AQI and AQC (in the absence of chloride) reveals that longer irradiation time is required for the former than for the latter. The Φ for cleavage of a GG step for AQC under these conditions has been determined⁶ to be 1.3×10^{-2} . It is important to note that cleavage by AQI is distributed throughout the DNA and that AQC-induced cleavage is localized. Consequently, comparison of irradiation time is not a direct measure of quantum efficiency for cleavage.

and protect shielded portions of the DNA from damage. Netropsin binds in the minor groove of DNA at sequences containing contiguous A or T bases.²¹ An autoradiogram of the restriction fragment irradiated in the presence of AQI and 0.5 μM netropsin shows clear high-resolution footprints of all the recognized AT binding sites and a previously unrecognized netropsin binding at an AAA sequence.¹²

The functional characteristics of the anthraquinone photoreductases depend strongly on their structures. For quinones that intercalate in DNA, irradiation leads to rapid electron transfer, localization of the radical cation at a GG step, and, after treatment with piperidine, cleavage predominantly at the 5'-G.^{6d} The spectroscopic and analytical experiments performed with AQI indicate that it has a different binding mode and a different characteristic reactivity. AQI does not bind to DNA primarily by intercalation. Nonintercalative binding reduces the rate of electron transfer, since there is no direct π -electron overlap with the DNA bases. The spontaneous cleavage of DNA observed from the irradiation of AQI is indicative of hydrogen atom abstraction from a deoxyribose unit. Anthraquinones having a predominant $n\pi^*$ excited state configuration generally intersystem cross efficiently and abstract hydrogen atoms rapidly from suitable substrates. Readily abstracted hydrogen atoms on the deoxyribose units are accessible from the major and minor grooves of DNA. The ability of AQI to footprint netropsin suggests that it reacts, at least in part, from the minor groove.²² These findings show that simple structural change to the anthraquinone derivative has a profound effects on its binding and chemical properties. We are exploring the detailed reaction mechanisms and applications of these nucleases.

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Supporting Information Available: A plot of the change in viscosity for AQI and AQS, an image of DNA visualized by SFM, spectra recorded 20 ps after excitation of AQC and AQI bound to DNA, and autoradiogram of a 248 base pair restriction fragment cleaved by AQI showing netropsin footprints (5 pages). See any current masthead page for ordering and Internet access instructions.

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(22) Some excited rhodium complexes are able to footprint ligands in the minor groove by reaction in the major groove.^{2c}

(23) At 15 μM AQS, the average length = 3732 nm and represents a ca. 300 nm (8.8%, $\sim 4\sigma$) increase. Studies over the same concentration range of AQI give no measurable increase in length. At 15 μM , the ratio of quinone to *p*BBH is approximately 9×10^5 . Lengthening data obtained through SFM analysis may be conveniently fit to various models so that approximate binding parameters can be estimated. Fitting of the AQS data to a simple neighbor exclusion model provides a binding affinity of ca. $2 \times 10^5 \text{ M}^{-1}$. This value is consistent with that previously found for AQS using absorption and fluorescence titration experiments.^{6b} An exclusion number of 3 was obtained by fitting of the data and is consistent with that found for other anthracycline intercalators of similar size such as daunomycin.¹⁴