Anthracoquinone 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 sequences and to develop agents that image (footprint) DNA molecules bound to DNA. 5 We recently described a set of anthraquinone derivatives that act as photonucleases. 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 CT free solution gives selective cleavage at the 5'-G of GG steps that is revealed only after treatment of the DNA with hot piperidine. 7 In contrast, spontaneous (no piperidine treatment required), nonselective cleavage is observed when AQC is irradiated in a solution containing CT. 8c 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 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. AQC 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 AQC binds to DNA by intercalation or by association with a groove. A competitive ethidium bromide displacement method was used to evaluate the binding of AQC in PBS solution to DNA. The association constants for AQC with [poly(dA)/poly(dT)] and with duplex poly[d(GdC)] are 0.5 and 3.9 × 10^1 M^-1, respectively. These values are similar to that measured for AQC with poly-[d(GdC)]. 5b 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 AQC on the viscosity of calf-thymus DNA solutions. The results reveal an increase in viscosity characteristic of intercalators for AQS. In contrast, AQC 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 AQC 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 AQC 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 AQC 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, σ = 75 nm). Images acquired following immobilization of the plasmid from an AQS solution (15 µM) reveal lengthening of the DNA by 300 nm. 15 In contrast, images acquired following immobilization from an AQC solution reveal no measurable change in DNA length. Figure 1 shows these effects quantitatively. Competition with ethidium bromide verifies that AQC blocks the minor groove. 16

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

(4) For a general review of synthetic nucleases, see: (a) Sigman, D. S.; Mazumder, A.; Perrin, D. M. Chem. Rev. 1993, 93, 2295–2316.
(12) The chemical structures of AQS and AQC are given in Figure 1.
(14) See Supporting Information.
(17) Incubation of pBBH with 10 µM AQC 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 × 10^3 M^-1 and an exclusion number of 2 as previously determined by SFM assay.
In contrast, irradiation of AQS or AQC under these conditions that polymers may be due to differences in binding constant. We estimate under these conditions that longer irradiation time is required for the former than for the latter.

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 fragment cleavage that was cleaved by irradiation of AQI shows spontaneous cleavage with low quantum efficiency and essentially equal effectiveness at every nucleotide. 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 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. Netropsin is bound to poly(dA)/poly(dT) at 77 K. When bound to poly(dA)/poly(dT) or duplex poly-dG-dC, the phosphorescence of AQI is shifted 7 nm to higher energy and its intensity is reduced 24% and 62%, respectively. In contrast, the phosphorescence of AQS in frozen solution is completely quenched 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 ps. Similar irradiation of bound AQC shows the strong absorption of the quinone radical anion in less than 20 ps. The laser spectroscopy and the incomplete phosphorescence quenching for AQI show that the rate of electron transfer is slowed significantly when the quinine is not intercalated.

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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.