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DNA photocleavage and biological activity of a pyrene dihydrodioxin

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Abstract—A pyrene dihydrodioxin has been synthesized, shown to bind to duplex DNA by intercalation, and cleave the ϕX 174 supercoiled plasmid upon irradiation with UV light. This compound also exhibits cytotoxic activity at the micromolar range in a number of human cancer cell lines.

The development of synthetic molecules that bind to, modify or cleave DNA, and ultimately result in cell death has been under intense research for the last couple of decades.[1] DNA photocleavage agents comprise a unique group of agents and are defined in a review by Armitage as 'those compounds whose excited states can initiate a series of chemical reactions, which ultimately lead to nucleic acid cleavage'.[2] Several classes of DNA photocleavage agents are known, such as metal complexes, organometallic complexes, and organic compounds including enediynes, nitrosubstituted aromatics, halogen containing compounds, riboflavins, naphthalimide derivatives, and certain anthraquinone derivatives.[3]

In 1944, Mustafa and Schönberg reported that 9,10-phenanthrenequinone (PAQ) undergoes photocycloaddition with substituted olefins resulting in the formation of a dihydrodioxin (DHD).[4] Schönberg observed that the photoaddition proceeds with visible light and is reversible at high temperatures or when treated with concentrated sulfuric acid. It was subsequently discovered that the quinone can be regenerated with long wavelength UV irradiation.[5] This discovery makes DHD derivatives promising DNA photocleaving agents since highly reactive *ortho*-quinones can be masked as DHDs, delivered to the nucleic acid target in this benign state, and then unmasked by UV irradiation when bound to the targeted DNA site. In this context, we have previously reported the site specific cleavage of a nucleic acid target by a phenanthrene based DHD–DNA conjugate[6] and have extended these ideas to include several different phenanthrene based DHDs.[7] In this report, the binding of the highly reactive pyrene DHD (**1**) to DNA, its DNA photocleaving properties, and a brief study of the nonphotochemical toxicity of this compound is reported as a baseline study for future photochemical studies in living systems (Scheme 1).

The synthesis of **1** has been reported previously along with a proposed mechanism of quinone release.[8] In that work, it was shown that photochemical quinone release proceeded via formation of the radical cation of **1**. Since it has been established that a number of related polycyclic aromatic hydrocarbons are oxidized via their radical cations by P450,[9] it was of interest to see whether **1** might exhibit traditional cytotoxicity in the absence of light. Therefore, **1** was submitted to the National Cancer Institute for testing in its In Vitro Cell Line Screening Project.[10] Indeed, **1** exhibited micromolar activity in a variety of human cancer cell lines. A sample of the most susceptible cell lines is summarized in Table 1.

These results confirm that **1** does have cytotoxic properties. While the NCI does not irradiate cell lines, it should be noted that in previous studies with related DHDs, and other cell lines, we found that in all cases irradiation significantly enhances cell death.[11] While these uncorrelated results clearly do not establish a link between cell death, the photochemical/thermal release of *ortho*-quinones, and DNA cleavage, they do provide an intriguing set of properties that would seem to warrant further, and more systematic, study in living systems.

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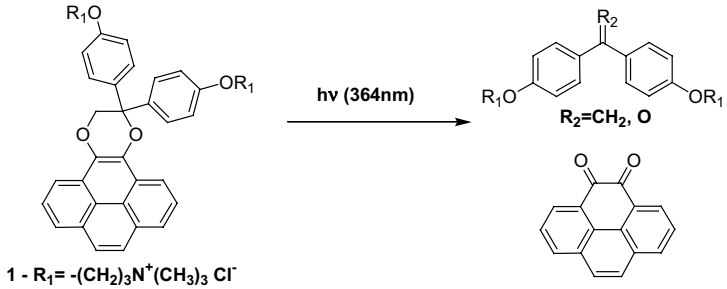

Scheme 1. Irradiation of **1** with the prism-selected 364 nm line of an argon ion laser releases pyrene quinone.

Table 1. Results of the most susceptible cell lines[a]

Cancer type	Cell line	GI50 (μM)	TGI (μM)	LC50 (μM)
Leukemia	K-562	5.68	46.3	>100
Non-small cell lung cancer	NCI-H322M	1.49	7.42	33.6
Colon cancer	HCT-116	4.07	20.7	50.3
CNS cancer	SF-268	1.38	6.69	34.1
Renal cancer	ACHN	4.47	21.6	72.3

[a] Results as obtained from the National Cancer Institutes In Vitro Cell Line Screening Project. A total of 50 cell lines were tested.

Toward this end, we have investigated the binding properties of **1** with DNA. The binding of **1** to single and double stranded DNA was examined by a variety of methods including equilibrium dialysis, UV–vis and fluorescence spectroscopy. After extensive investigation, the equilibrium dialysis method of Chaires was found to provide the best estimation of an association constant for **1** with dsDNA.[12] This method does not suffer from the problems of aggregation and light scattering at high drug to DNA ratios as do the optical methods. Repeated dialysis experiments resulted in an estimation of K_{assoc} $(7.0 \pm 1.3) \times 10^5$ in units of base pairs. This value is consistent with strong binding and intercalation.

Titrating a solution of **1** with aliquots of calf thymus DNA results in a hypochromic shift in the UV–vis spectrum, which is an indication of intercalation and a bathochromic shift, which indicates a strong association between **1** and the DNA (Fig. 1).

The association between **1** and DNA was examined further by an iodide fluorescence quenching study. A Stern–Volmer plot of **1** in the presence and absence of DNA clearly shows that **1** is sheltered from the quencher, and therefore, bound to the DNA (Fig. 2).

Fluorescence spectroscopy shows that **1** is highly fluorescent and the emission spectrum is shifted to longer wavelength by the addition of DNA (Fig. 3). The presence of an isosbestic point is strong evidence for the presence of only two emitting species, namely the bound and unbound DHD. It is thought that the new band formed in the emission spectra is exciplex emission due to the strong association between the DNA bases and the intercalated excited state of the pyrene.[13]

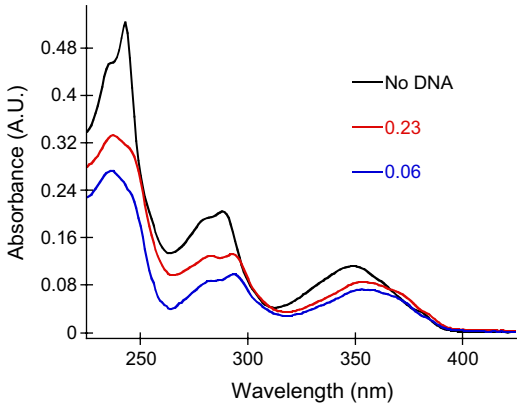

Figure 1. Absorption spectra of **1** upon addition of calf thymus DNA. The black line is the spectrum in the absence of DNA, the red line at [DHD]:[DNA]bp = 0.23, and the blue line at [DHD]:[DNA]bp = 0.06.

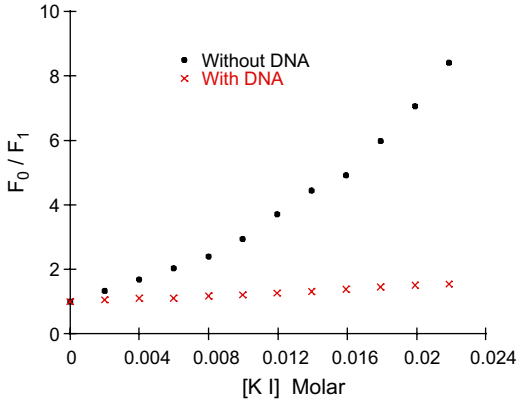

Figure 2. Stern–Volmer plot showing the fluorescence quenching of **1** by potassium iodide in the absence and presence of DNA (λ excitation = 350 nm).

A fluorescence resonance energy transfer (FRET) study was conducted at low drug to base pair ratios in order to confirm the binding mode (Fig. 4). The fluorescence quantum yield of bound **1** is increased at 260 nm in the presence of dsDNA and decreased in the presence of pre-formed triplex DNA. The complex of **1** and single stranded DNA yields values in between the duplex and triplex around 260 nm. This effect is further evidence toward intercalation as the predominant binding mode for dsDNA.[14]

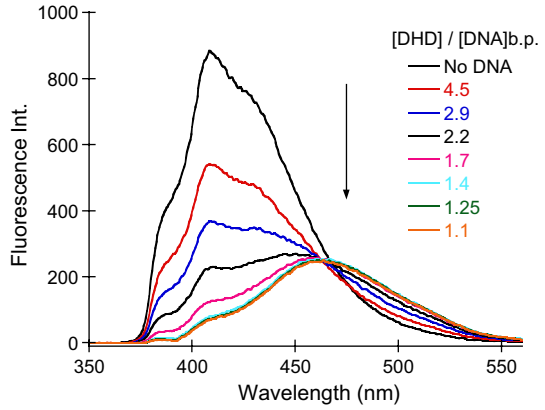

Figure 3. Fluorescence spectra (λ excitation = 350 nm) of **1** upon addition of calf thymus DNA. The numbers correspond to [DHD]:[DNA]bp.

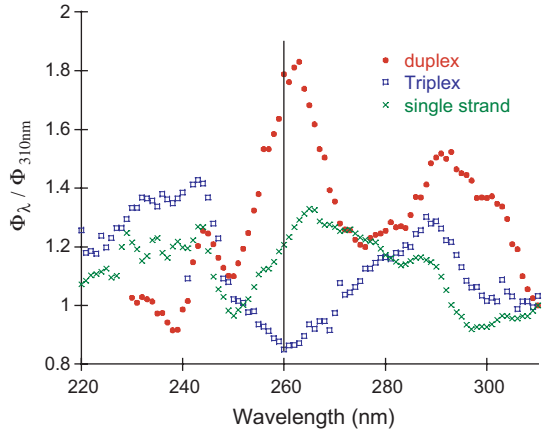

Figure 4. Fluorescence resonance energy transfer plot of **1** with duplex (●), single stranded poly-T (×), and pre-formed triplex (□) DNA.

The photocleaving of DNA by **1** was tested using the supercoiled plasmid relaxation assay. Upon 364 nm irradiation, **1** both nicked (form II) and linearized (form III) the ϕX 174 supercoiled plasmid (form I) (Fig. 5). The presence of all three forms of DNA (forms I–III) in the reaction mixture is an indication of nonrandom cleavage events.[15] The ratios of single stranded cuts (n_1) to double stranded cuts (n_2) per molecule provides an estimate of the randomness of DNA cleavage, (n_1/n_2). The value for the single strand cuts per molecule (n_1) was calculated from the fraction of the nicked, form II, and the number of double-strand cuts (n_2) from the fraction of linear form, form III. The single-strand to double-strand cut ratios were in the range 14–29 (Table 2).

These values are much lower than the expected ratio for strictly random single-strand cleavage events, which for ϕX 174 with 5386 base pairs would require >750 cuts for a single linearization to occur.[16] These data indicate that double-strand cleavage is particularly favorable with **1**. Thus, once released the quinone seems to be strongly associated with the DNA, and can inflict multiple lesions in the DNA backbone in the region of its release.[17]

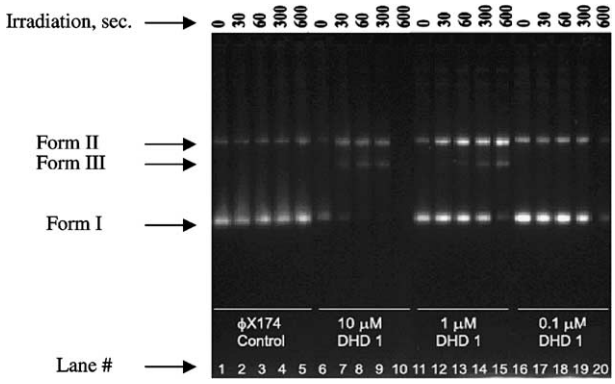

Figure 5. Photocleavage of supercoiled ϕX 174 plasmid. The cleavage efficiency was evaluated using the supercoiled ϕX 174 plasmid (form I) (0.33 μg/10 μL) with **1** in Tris–acetate EDTA buffer (pH 7.6). Each sample was irradiated with the 364 nm line of an Ar ion laser while maintaining the sample at 3C then analyzed on a 1.2% agarose gel. Lanes 1–5, DNA alone with increasing irradiation time of 0–600 s; lanes 6–10 10 μM of **1** (1DHD:1bp) with increasing irradiation time of 0–600 s; lanes 11–15 1.0 μM of **1** (1DHD:50bp) with increasing irradiation time of 0–600 s; lanes 16–20 0.1 μM of **1** (1DHD:500bp) with increasing irradiation time of 0–600 s.

Table 2. Statistical efficiency of single-strand (n_1) and double-strand (n_2) cleavage by **1**[a]

Compound	Concn (μM)	Irradiation time (s)	n_1	n_2	n_1/n_2
1	1	240	1.13	0.04	28.5
1	1	420	1.45	0.08	18
1	1	600	1.45	0.1	14.5

[a] The number of single-strand cuts and double-strand cuts were determined using the statistical test of Provirk et al.[16]

In conclusion, it has been shown that **1** has micromolar activity in the NCI's In Vitro Cell Line Screening Project. Compound **1** binds to duplex DNA by intercalation and damages plasmid DNA upon photochemical initiation. We are currently investigating the mechanism of photocleavage, the optimization of the DNA cleaving efficiencies of quinones derived from polycyclic aromatic hydrocarbons, and the extension of these studies to living systems.

Acknowledgements

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