Articles

Photoprocesses of Naphthalene Imide and Diimide Derivatives in Aqueous Solutions of DNA

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Abstract: Despite the growing number of redox-active chromophores utilized to photoinduce oligonucleotide cleavage, detailed correlations between the degree of ground-state complexation and product yields have not been developed. To elucidate the specific role of singlet and triplet excited states in nucleotide photooxidation, the photochemical reactivities of *N*-(2-(*N*-pyridinium)ethyl)-1,8-naphthalene imide (NI) and *N*,*N*′-bis-[2-(*N*-pyridinium)ethyl]-1,4,5,8-naphthalene diimide (NDI) with calf-thymus DNA have been explored as a function of ground-state complexation with the DNA polymer. Upon addition of calf-thymus DNA to a phosphate buffered solution of the naphthalene imide derivatives, distinct changes in the UV absorption spectrum of the chromophores, along with single isosbestic points, are observed. Analysis of these changes using the noncooperative model of McGhee and von Hippel yield association constants of $(2.46 \pm 0.42) \times 10^4$ M^{-1} and $(7.78 \pm 0.11) \times 10^5$ M^{-1} for NI and NDI, respectively. Pulsed 355 nm excitation of either NI or NDI in the presence of calf-thymus DNA produced the reduced $NI^{\bullet -}$ and $NDI^{\bullet -}$ species that absorbed maximally at 400 and 480 nm, respectively, from the triplet excited states. For both compounds, the yield of radical anion from self-quenching processes was substantial ($\phi_{I^{\bullet -}} = 0.11 \pm 0.01$ and 0.25 ± 0.01 for NI and NDI, respectively). However, pulsed excitation of NI in the presence of DNA resulted in the production of radical species that were not attributed to self-quenching processes. For both compounds, the fraction of associated imide was systematically varied between 0 and 1. The intersystem crossing yield was found to decrease linearly with the fraction bound to DNA from 0.71 to 0.08 for NI and 0.35 to 0.004 for NDI.

Introduction

The enormous interest in the development of chemical systems as structural probes in biological macromolecules, as well as therapeutic agents, has led to the design of a growing number of studies on synthetic "reagents" that can be activated by UV or visible light. The ability to deliver chemical reactivity on demand has been used in photodynamic therapy, where the localized interaction of a chromophore and light results in tumor destruction. Recently, the development of synthetic chemical systems that can photocleave nucleic acid or protein polymers has emerged into a growing database of molecular systems capable of photoinitiating specific or nonspecific lesions. This work is largely driven by the desire to create molecular chromophores as (a) nonsequence-specific reagents for utilization in photofootprinting experiments, or (b) sequence-specific structural probes and sequencing agents.

Of the chromophores employed, hydroxyl radical generators, singlet oxygen generators, electron transfer agents, and excited states capable of hydrogen atom abstraction from the nucleotide ribose unit have been most studied. The multitude of synthetic systems used for photoinitiated oligonucleotide cleavage has been recently reviewed.[1] Desirable chemical systems are those that demonstrate a versatile range of photochemical reactivity that can be tuned for specific or nonspecific interactions. This versatility may be achieved by modification of a specific chemical system to (a) favor site-specific association and reactivity or (b) preferentially produce nonselective and diffusible intermediates (e.g. hydroxyl radicals). The naphthalene-derived imide and diimide chromophores have been shown to be a class of chromophores capable of generating a multitude of reactive intermediates.[2−5] In a previous report, we have shown that the triplet excited states of 1,8-naphthalimide and 1,4,5,8-naphthaldiimide derivatives are photoreduced by the individual nucleotides and that the kinetics of nucleotide oxidation are tunable, over ∼2 orders of magnitude, depending upon the nucleotide employed.[6] This observation, coupled with the prospects of readily functionalizing (on the imide nitrogen) the naphthalene-derived imides with specific recognition groups, makes them ideal candidates for sequence-specific photooxidation and cleavage of oligonucleotide polymers.

Specifically, L-lysine derivatives of 1,8-naphthalimide have been previously reported to nick both supercoiled DNA, as well

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as double-stranded DNA fragments, upon UV irradiation and treatment with hot piperidine.[4] Specific cleavage at the 5′-side of 5′-GG-3′ steps was observed. Upon nitration of the naphthalimide ring, photocleavage at thymine sites was also observed. Variable selectivity between these two modes of cleavage was obtained, depending upon the position of the nitro substituent. In a subsequent publication, Saito et al. demonstrated that irradiation of this L-lysine 1,8-naphthalimide derivative in the presence of a duplex hexamer selectively produced piperidine-labile damage at the 5′-side of 5′-GG-3′ sites.[5] Using laser flash photolysis, the one-electron reduced imide was directly detected. Production of this species was via bimolecular quenching of the naphthalimide triplet state. The quenching rate constant was estimated to be 5.3×10^7 M^{-1} s^{-1}. When the photoinduced reaction is not base-specific and not reversible, site-specific damage is rendered only when the chromophore is tightly associated with the target of interest. This prerequisite localizes the site of damage and results in rapid (since the process is not diffusion controlled) photochemical reactions. Moreover, rapid excited state quenching by the associated target precludes the formation of diffusible species (e.g., singlet oxygen). Although the naphthalimide derivatives are flat planar species, likely to intercalate between DNA base pairs, no evidence of ground-state complexation was indicated in the reports of Saito et al.[4,5]

In a series of separate reports, the noncovalent interactions of a series of *N*-alkylamine-substituted naphthalene imides with DNA were assessed.[7−10] Since the pendant alkylamine is protonated in neutral aqueous solution, the binding constants were shown to be strongly dependent upon ionic strength.[9] For a given DNA substrate, imide substituent, and ionic strength (0.10 M NaCl), the binding constants for the naphthaldiimide derivatives ($K = (3.0-4.6) \times 10^5$ M^{-1}) were ∼10-fold larger than those of the corresponding naphthalene monoimide.[7]

The ability of functionalized naphthalene imides and diimides to strongly associate with DNA and, in certain cases, exhibit a binding preference for GC-rich regions,[10] makes them viable candidates to explore the photoredox chemistry while associated to DNA. A detailed understanding of the photochemical mechanisms must first consider the nature of the excited states responsible for damage. Under conditions where the chromophore is not associated with the DNA polymer, photodamage initiated by triplet states will predominate, owing to their long lifetimes. However, upon association of the chromophore to the target, rapid nucleotide oxidation by the singlet excited-state becomes competitive. Triplet-state quenching of a naphthalene imide by oligonucleotide duplexes has been demonstrated.[5] However, the partitioning of singlet and triplet state reactivity, while associated with the oligonucleotides, has not been explored.

We have previously demonstrated that the triplet states of naphthalene imide and diimide systems do oxidize individual nucleotides.[6] However, nucleotide oxidation in DNA polymers by these imide excited states has not yet been demonstrated. The reports by Saito et al. provide convincing evidence that the triplet excited states of 1,8-naphthalimide derivatives does, indeed, produce oxidized nucleotide, with subsequent oligonucleotide cleavage. However, the role of the singlet excited state remains unclear in cases where the chromophore is associated with the DNA polymer. Moreover, the photoredox activity of the excited states of the corresponding naphthaldiimide chromophore with DNA has not been characterized. Although a number of organic and inorganic chemical systems have been employed to photochemically cleave oligonucleotide polymers, the photochemical efficiency has not been systematically correlated with extent of ground-state complexation. In this paper, we (a) report the synthesis of a novel pair of cationic naphthalene imide and diimide derivatives, (b) characterize the noncovalent interactions of these two photoredox reagents with calf-thymus DNA, and (c) demonstrate the first systematic investigation of the relative roles of singlet and triplet excited states in nucleotide oxidation.

Experimental Section

Materials. 2-Bromoethylamine hydrobromide, 1,4,5,8-naphthalenetetracarboxylic dianhydride, ethanolamine (99+ %), *p*-toluenesolfonyl chloride (99+%), methyl viologen dichloride hydrate (98%), 1,4-diazobicyclo [2.2.2]-octane (DABCO) (98%) and benzyl viologen dichloride (97%) were used as received from Aldrich Chemicals (Milwaukee, WI). 1,8-naphthalic anhydride (97%, Acros) was recrystallized from *N*,*N*-dimethylacetamide (DMA) and dried in vacuo prior to use. Sodium hydrogen phosphate (99%) was obtained from Acros and used as received. Solutions of calf-thymus DNA (highly polymerized sodium salt (Sigma, St. Louis, MO)) were sonicated at 25 °C for 1 h. The resulting solution was filtered through a 0.45 μM Millipore filter. DNA concentrations (in base pairs) were determined spectroscopically using $\epsilon = 13\,200$ M^{-1} cm^{-1} at 260 nm.[11] DNA stock solutions were stored at 4 °C and discarded after one week.

Water was deionized and freshly passed through an Ion-Pure Reverse Osmosis system. The system utilizes a point of use cartridge system, followed by UV irradiation to provide > 18 MΩ ultrapure bacteria-free water. Other materials were obtained from commercial sources.

***N*-(2-(*N*-Pyridinium)ethyl)-1,8-naphthalene Imide (NI, 1).** Ethanolamine (5 mL) was added dropwise over 15 min to a solution of 1,8-naphthalic anhydride (3.46 g, 17.4 mmol) in 40 mL of DMA. The mixture was heated at 100 °C for 2 h. The crude reaction mixture was concentrated on a rotary evaporator,and the product washed with 95% ethanol. The resulting *N*-(2-ethanol)-1,8-naphthalimide (3.16 g, 13.1 mmol) and *p*-toluenesulfonyl chloride (2.75 g, 14.4 mmol) were stirred in pyridine at room temperature for 20 h and then refluxed for 5 h. After conversion to the chloride salt (DOWEX 1 × 8−200 ion-exchange resin), the crude product was recrystallized in methanol under an atmosphere of acetone. ^{1}H NMR (DMSO-d_6): 9.20 (d, 2H, pyr), 8.60 (t, 1H, pyr), 8.40 (d+d, 4H, napht), 8.05 (t, 2H, pyr), 7.80 (t, 2H, napht), 4.95 (t, 2H, $-CH_2$), 4.60 (t, 2H, $-CH_2$). Anal. Calcd C, 67.36, H, 4.43, N, 8.27. Found C, 66.57, H, 4.43, N, 8.15. UV max (10 mM phosphate buffer; pH 7.00) 344 nm ($\epsilon = 13\,500$ M^{-1} cm^{-1}); 264 nm (4800 M^{-1} cm^{-1}); 232 nm ($\epsilon = 38\,900$ M^{-1} cm^{-1}); 214 nm ($\epsilon = 21\,700$ M^{-1} cm^{-1}).

***N*,*N*′-bis-[2-(*N*-Pyridinium)ethyl]-1,4,5,8-naphthalene Diimide (NDI, 2).** Ethanolamine (10 mL) was added dropwise (over a period of 15 min) to a solution of 1,4,5,8-naphthalenetetracarboxylic dianhydride (5.00 g, 18.6 mmol) in 40 mL *N*,*N*-dimethylformamide (DMF). The reaction mixture was heated at 90 °C for 2 h. The resulting *N*,*N*′-(2-ethanol)-1,4,5,8-naphthalene diimide **(3)** was filtered, and the precipitate was washed with acetone. Compound **(3)** (2.3 g, 9.02 mmol) and *p*-toluenesulfonyl chloride (3.78 g, 19.84 mmol) were stirred in pyridine at room temperature for 20 h and then refluxed for 5 h. The product was filtered, and the precipitate was washed with acetone and recrystallized from methanol. ^{1}H NMR (DMSO-d_6): 9.24 (d, 4H, pyr), 8.61 (t + s, 6H, pyr + napht), 8.09 (t, 4H, pyr), 4.96 (t, 4H, $-CH_2$), 4.62 (t, 4H, $-CH_2$). Anal. Calcd (for NDI·$2H_2O$) C, 57.44, H, 4.44, N, 9.57. Found C, 57.10, H, 4.41, N, 9.51. UV max (10 mM phosphate buffer; pH 7.00): 382 nm ($\epsilon = 25\,600$ M^{-1} cm^{-1}), 362 nm ($\epsilon = 21\,000$ M^{-1} cm^{-1}), 260 nm ($\epsilon = 9720$ M^{-1} cm^{-1}), 234 nm ($\epsilon = 35\,200$ M^{-1} cm^{-1}).

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Ground-State Complexation Studies. A stock solution of sonicated calf thymus DNA (1−4 mM base pairs in H_2O) was added in 10 μL aliquots (not to exceed 200 μL) to 2.00 mL of NI or NDI solution (10−30 μM in 10 mM phosphate buffer at pH = 7.0). Ground-state spectral changes were monitored as a function of added DNA. Spectral changes were analyzed as described below at 344 and 354 nm for NI or 362 and 382 nm for NDI. Data from a total of three (for NI) or five (for NDI) experiments were analyzed.

The extinction coefficient of the free imide (ϵ_f) was determined through a Beer's Law plot, and the extinction coefficient of the bound complex (ϵ_b) was estimated by dividing the limiting absorbance by the imide concentration. From the spectral changes ($\Delta A = (A_o - A/A_o)$) and extinction coefficient data, the binding density ν (concentration of bound imide/total DNA concentration) was calculated according to eq 1.[12]

$$\Delta A\left(\frac{L_T}{M_T}\right) = \left(\frac{\epsilon_b - \epsilon_f}{\epsilon_f}\right)\nu \quad (1)$$

where L_T and M_T are the total concentrations of imide and DNA, respectively. The concentration of "free" imide (L_f) was calculated according to eq 2.

$$L_f = L_T - \nu M_T \quad (2)$$

For each wavelength that was analyzed, plots of ν/L_f vs ν were constructed, and the curves were fit to the noncooperative model of McGhee and von Hippel.[13] Values of K (equilibrium constant in M^{-1}) and n (number of base pairs associated with each bound imide) were determined by a nonlinear least-squares regression. The average and standard deviation for each set of data were calculated. At each imide/DNA ratio employed, the fraction of imide molecules not associated with the DNA polymer ($f_1 = L_f/L_T$) was evaluated from the ground-state spectral changes associated with the DNA addition.

General Techniques. Laser flash photolysis kinetic investigations were carried out at 22.0 ± 0.2 °C using a water-jacketed sample holder connected to a Lauda RM6-B circulating water bath. Ground-state UV/vis absorption spectra were measured using a JASCO V-570 double-beam spectrophotometer. Proton NMR spectra were obtained using either a GE QE−300 or a Varian Mercury 200 MHz NMR spectrometer. Fluorescence spectra were measured using a SPEX Fluoromax-2 fluorescence spectrometer. Excitation wavelengths of 368 nm and 390 nm were used for NI and NDI, respectively.

Nanosecond transient absorption measurements employed the technique of laser flash photolysis. The third-harmonic (355 nm) of a Q-switched Nd:YAG laser (Continuum Surelight II, pulse width ~8 ns) was used for laser flash excitation. Pulse energies of up to 25 mJ cm^{-2} $pulse^{-1}$ were typically employed. A detailed description of the laser flash photolysis apparatus has been previously published.[6]

One-electron reduction potentials of the imide and diimide were measured in 0.10 M Na_2SO_4 aqueous solution containing ~5 mM imide. Solutions were bubbled with nitrogen prior to measurement. The cyclic voltammograms were obtained using a BAS CV-1B CV controller that was interfaced to a pentium PC for data acquisition. For these measurements, platinum auxiliary and glassy-carbon working electrodes were employed, with a silver/silver chloride (approximately 3 M KCl) reference electrode. The electrochemical half-wave potentials of the imide, measured with respect to the Ag/AgCl reference electrode, were converted to a NHE reference using $E^\circ_{1/2} = 0.36$ V vs NHE for the $Fe(CN)_6^{3-/4-}$ couple in ferrocyanide.[14] A scan rate of 100mV/sec was typically employed.

Results

Photophysical and Redox Properties. Shown in Figure 1 are the structures of the naphthalene imide (NI, **1**) and diimide (NDI, **2**) derivatives employed in this study. The pyridinium derivatives of the chromophores were prepared to provide water soluble photooxidizing agents that can noncovalently associate to double stranded DNA, either by an intercalative or electrostatic mode. In both cases, the ground-state absorption spectra of NI and NDI exhibit the $S_0 - S_1$ vibronic progression at wavelengths longer than 300 nm. Higher energy $\pi-\pi^*$ transitions are observed at wavelengths shorter than 250 nm. The one-electron reduction potentials were determined using cyclic voltammetry. The half-wave potentials for the reduction of NI and NDI were determined to be −0.84 and −0.11 (V vs NHE), respectively.

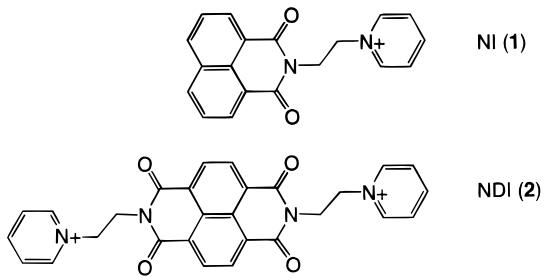


Figure 1. Structures of NI (1) and NDI (2) naphthalene imide derivatives employed in this work.

Upon 355 nm nanosecond pulsed excitation of a deaerated solution of **1** or **2**, ground-state bleaching is observed concomitant with the formation of transients absorbing at longer wavelengths (Figure 2). In both cases, the transients were quenched by molecular oxygen. The transient absorption spectra are identical to the $T_1 - T_n$ spectra reported previously for other naphthalene imide and naphthalene diimide derivatives.[2,6] At low excitation energies, the triplet decay kinetics are first-order. At higher excitation energies, triplet−triplet annihilation is observed. Moreover, at high NI or NDI concentration, self-quenching was observed ($k_{self} \approx 1.2 \times 10^9$ M^{-1} s^{-1} and 2×10^9 M^{-1} s^{-1} for NI and NDI, respectively). Under conditions of low excitation intensities and small ground-state concentrations, the triplet state lifetimes of NI and NDI were found to be 162 and 100 μs, respectively in deoxygenated 10 mM pH 7.00 phosphate buffer (Figure 2 insets). The triplet-state lifetimes in aqueous buffer solutions were somewhat longer than those reported previously for neutral NI and NDI excited states in 1:1 $CH_3CN:H_2O$.[6]

Spectra of Reduced NI and NDI. To characterize the UV/vis spectrum of the one-electron reduced form of the naphthalene imide (**1**) and diimide (**2**) derivatives, DABCO was used as a reductive quencher of the imide and diimide triplet states. Under conditions where the imide triplet state is exclusively and quantitatively quenched by DABCO, reduced NI or NDI is observed concomitant with triplet-state decay. Production of $NI^{\bullet-}$ or $NDI^{\bullet-}$ in the presence of a secondary electron acceptor resulted in the growth of the reduced acceptor.[15] In these investigations, methyl viologen and benzyl viologen were employed as secondary electron acceptors from $NI^{\bullet-}$ and $NDI^{\bullet-}$, respectively. Since the one-electron reduction potential of methyl viologen in water ($E^\circ_{1/2} = -0.450$ V vs NHE)[16] is more negative than that for the $NDI/NDI^{\bullet-}$ couple, benzyl viologen was used as a better elec-

(12) Lohman, T.; Bujalowski, W. Thermodynamic Methods for Model-Independent Determination of Equilibrium Binding Isotherms for Protein-DNA Interactions: Spectroscopic Approaches to Monitor Binding. In *Methods in Enzymology*; Sauer, R. T., Ed.; Academic Press: New York, 1991; Vol. 208, pp 258−290.

(13) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469−489.

(14) Sawyer, D. T.; Sobkowiak, A.; Roberts, J. L. *Electrochemistry for Chemists*, 2nd ed.; Wiley-Interscience: New York, 1995; p 27.

(15) In these experiments, the concentration of the secondary electron acceptor was kept small enough (50 μM for methyl and benzyl viologen) in relation to DABCO ([DABCO] = 6.2 mM and 10 mM for NI and NDI, respectively) to preclude direct quenching of the imide triplet state by the bipyridine derivatives. Under these conditions, the imide radical is quantitatively "scavenged" by the viologen.

(16) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637−1723.

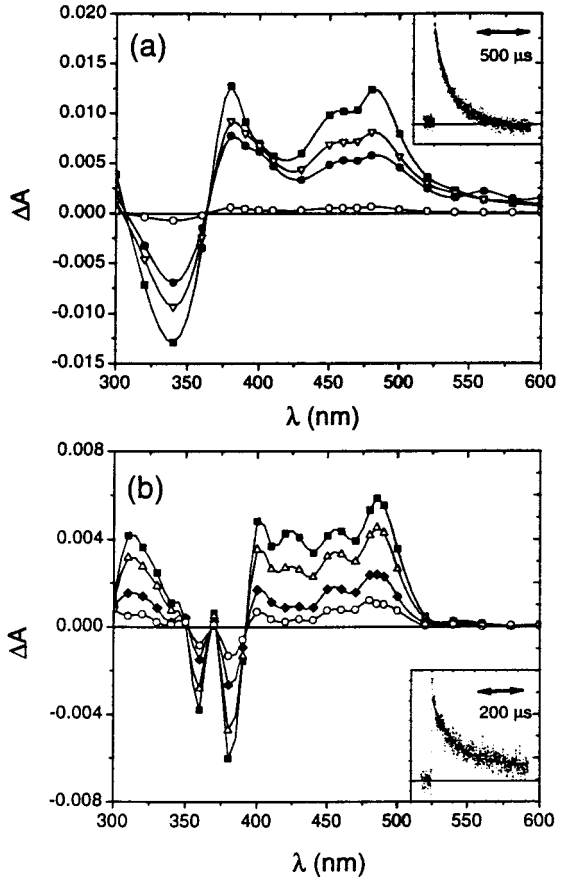


Figure 2. $T_1 - T_n$ absorption spectra observed after pulsed (8 ns pulse) 355 nm excitation of (a) NI ([NI] = 5 μM) and (b) NDI ([NDI] = 1 μM) in 10 mM phosphate buffer (pH 7.0) under argon-saturated conditions. Times shown are (a) 5, 50, and 100 μs after the laser pulse and (b) 0, 50, 100, and 300 μs after the laser pulse. Insets: Triplet decay traces with first-order fits measured at (a) 480 nm and (b) 400 nm.

tron-accepting agent ($E^{\circ}_{1/2} = -0.360$ V vs NHE).[16] The concentration of reduced methyl or benzyl viologen was determined from the absorbance change (extrapolated to time zero) at 395 or 603 nm, respectively.[17,18] The extinction coefficients of the imide radical anions were then determined from their respective absorption changes (at time zero after the laser pulse) following quenching of the triplet precursor by DABCO. The spectra of $NI^{\bullet-}$ and $NDI^{\bullet-}$ are shown in Figure 3.

Redox Products Produced from Self-Quenching. To elucidate the products from the self-quenching process, transient absorption spectra were measured under conditions where significant quenching of ^{3}NI* and ^{3}NDI* by their respective ground states does occur. The transient spectral features of the products are identical with those of $NI^{\bullet-}$ and $NDI^{\bullet-}$. Our observation of single-electron transfer from self-quenching is consistent with that reported by Aveline et al. when a naphthalene diimide derivative was used.[2] Although the oxidized species are necessarily produced, the similarity of the self-quenching spectra to those shown in Figure 3 suggests that these species absorb weakly in the wavelength range that was probed.

Using the extinction coefficients of $NI^{\bullet-}$ and $NDI^{\bullet-}$ from Figure 3, the yields of these radical anions produced from self-quenching ($\phi_{I^{\bullet-}}$) were determined from eq 3.

(17) Watanabe, T.; Honda, K. *J. Phys. Chem.* **1982**, *86*, 2617−2619 ($\epsilon^{V^{\bullet+}}_{395nm}$ = 42 100 M^{-1} cm^{-1}).

(18) Tsukahara, K.; Wilkins, R. *J. Am. Chem. Soc.* **1985**, *107*, 2632−2635 ($\epsilon^{V^{\bullet+}}_{395nm}$ = 14 000 M^{-1} cm^{-1}).

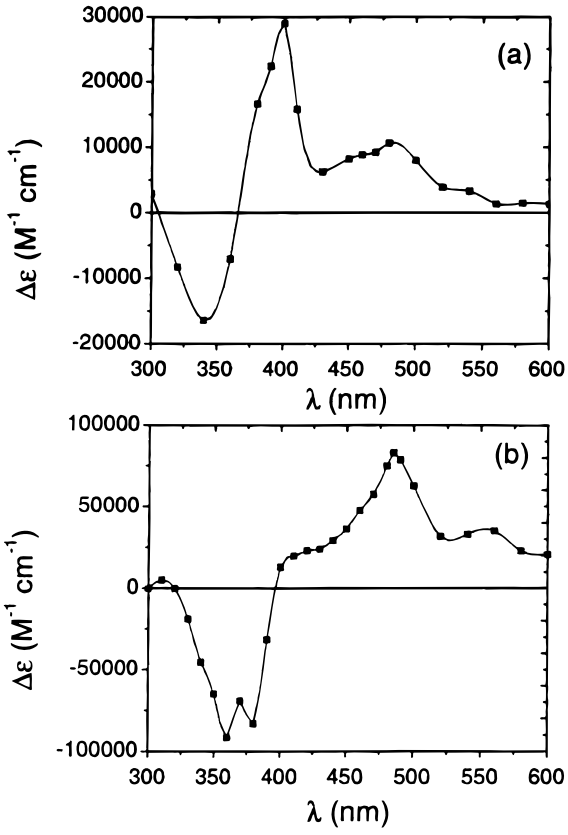


Figure 3. Transient absorption spectra of imide radical anions observed upon 355 nm excitation of (a) 5 μM NI containing 520 μM DABCO and (b) 11 μM NDI containing 1.67 mM DABCO. All solutions were argon-saturated and contain 10 mM pH 7.0 phosphate buffer.

$$\Delta A_{I^{\bullet-}} = \Delta\epsilon_{I^{\bullet-}}[^3I^*]_o f_q \phi_{I^{\bullet-}} \quad (3)$$

In eq 3, $\Delta A_{I^{\bullet-}}$ is the transient absorbance from the one-electron reduced imide, obtained by extrapolating the decay component to time zero, $\Delta\epsilon_{I^{\bullet-}}$ is the extinction coefficient of the radical, $[^3I^*]_o$ is the triplet state concentration produced by the laser pulse,[19] and $f_q = (k_{self}[I]/k_{self}[I] + k_d)$ is the fraction of triplet states quenched by the ground state of the imide. The absorption change observed at 400 and 480 nm, respectively, for NI and NDI was plotted as a function of $[^3I^*]_o f_q$. Fits of the data to eq 3 (see Supporting Information) yield $\phi_{I^{\bullet-}}$ of 0.11 ± 0.01 and 0.25 ± 0.01 for NI and NDI, respectively.

Ground-State Interactions with Calf Thymus DNA. Upon addition of calf-thymus DNA to a buffered solution of NI or NDI, a decrease in the long-wavelength absorption band intensity and slight red-shift are observed (Figure 4). In a and b of Figure 4, a single clean isosbestic point is observed at all imide/DNA ratios, suggesting that only one spectrally distinct imide/DNA complex is present. The spectral changes were analyzed and fit to the noncooperative model of McGhee and von Hippel,[13] to obtain the binding constant and average number of occupied sites in the DNA polymer. Analysis of the observed spectral changes with added calf thymus DNA yielded binding constants of (2.46 ± 0.42) × 10^4 M^{-1} and (7.78 ± 0.11) × 10^5 M^{-1} for NI and NDI, respectively. In both cases, the average occupied site size was determined to be 2.7 base pairs.

In both cases, a ~60% hypochromicity[20] is observed upon complexation to DNA. Moreover, a 5−10 nm red-shift is

(19) The triplet-state concentration was evaluated from the T − T absorption change at 480 nm for both NI and NDI.

(20) Percent hypochromicity calculated from the decrease in molar extinction coefficient upon binding to DNA ($(\epsilon_f - \epsilon_b/\epsilon_f)$).

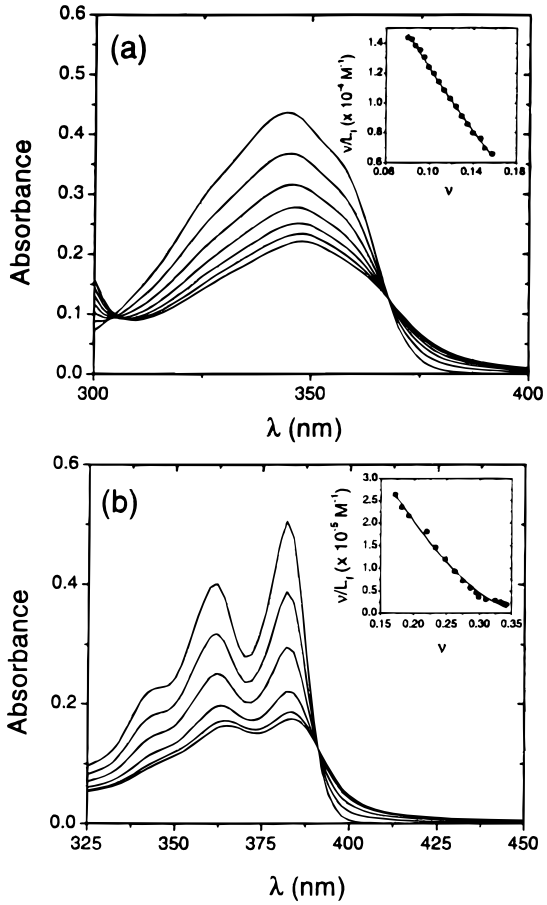


Figure 4. Ground-state spectral changes observed upon addition of calf thymus DNA to (a) 32 μM NI (0−340 μM DNA) and (b) 21 μM NDI (0−110 μM DNA) in 10 mM pH 7.0 phosphate buffer. Insets: Data from (a) 344 nm and (b) 382 nm analyzed according to eqs 1 and 2 and fitted to the noncooperative model of McGhee and von Hippel.

observed for both compounds. Both observations are qualitatively consistent with those observed for intercalating compounds. Using cationic naphthalene imide and diimide derivatives that are nearly identical to those employed in this work, the mode of association with calf thymus DNA has been previously investigated and found to be exclusively intercalative.[21] The ground-state spectral changes observed in our work are identical with those reported by Yen et al.. Thus, we assume that the primary mode of DNA interaction with NI and NDI is predominantly that of intercalation.

Excited-State Interactions with Calf Thymus DNA. Upon 355 nm laser flash excitation of a dearated aqueous buffered solution of NI (**1**) containing calf thymus DNA, a transient is observed immediately after the laser pulse that absorbs maximally at 480 nm (Figure 5a). The spectral features of this transient are consistent with those of the NI triplet state shown in Figure 2a. With time, the triplet state evolves to produce a second, long-lived transient. By comparison of the spectrum of this species with that of the radical anion shown in Figure 3a, we have identified this species as the one-electron reduced form of NI.

Since self-quenching of nonassociated ^{3}NI* molecules by NI does occur, we considered the possibility that the radical anion

(21) The mode of binding of cationic naphthalene imide and diimide derivatives (the pendant groups on the imide nitrogens are a quaternary alkylammonium substituents) to DNA has been reported in ref 7. Using a combination of viscometric titrations, along with circular dichroism and UV/vis spectroscopies, Yen et al. have shown that the compounds interact strongly with DNA through intercalative interactions.

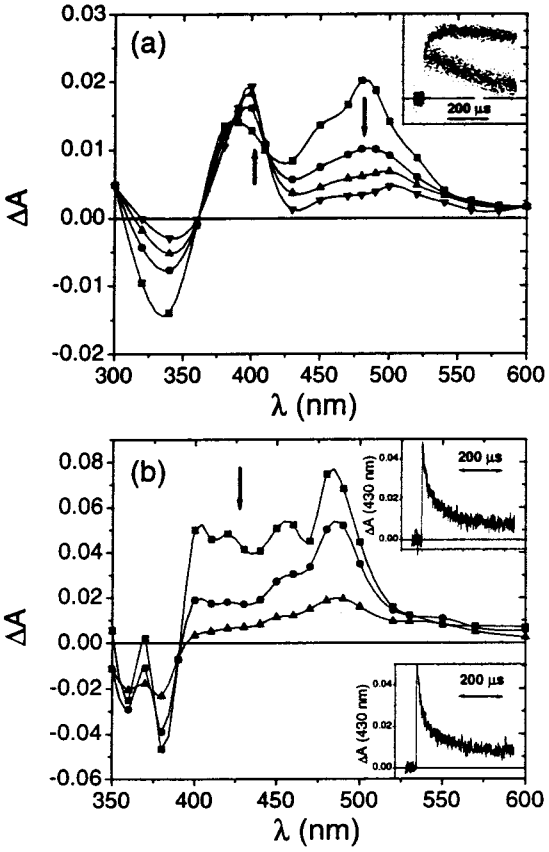


Figure 5. (a) Transient absorption spectra observed after pulsed 355 nm excitation of 42 μM solution of NI in the presence of 202 μM calf thymus DNA. Times shown are 0, 23, 53, and 152 μs after the laser pulse (decreasing ΔA at 480 nm). Inset: Top trace shows exponential growth of NI$^{\bullet-}$ (monitored at 400 nm) in the presence of DNA. Bottom trace shows the biexponential growth and decay of NI$^{\bullet-}$ produced from self-quenching (no added DNA). In this case, the concentration of NI = 5.5 μM in buffer corresponds to the concentration of "free" imide when DNA is present. (b) Transient absorption spectra observed after pulsed 355 nm excitation of 30 μM solution of NDI in the presence of 30 μM calf thymus DNA. Times shown are 0, 50, and 300 μs after the excitation pulse (decreasing ΔA at 485 nm). From the measured ground-state absorption spectra, along with eq 1, data shown in (a) and (b) correspond to 87 and 46% of NI and NDI, respectively, associated with the DNA All solutions are argon-saturated and in 10 mM phosphate buffer (pH 7.0). Inset: Biexponential formation and decay of NDI$^{\bullet-}$ (monitored at 430 nm) in the presence of DNA (top trace). Bottom trace shows the biexponential formation and decay of NDI$^{\bullet-}$ produced from self-quenching (no added DNA). In this case, the concentration of NDI = 16 μM and corresponds to the concentration of "free" imide when DNA is present.

observed could be arising from dynamic self-quenching.[22] To test this hypothesis, a buffered solution of NI was prepared at a concentration corresponding to that of the nonassociated ("free") imide in the solution containing DNA. Shown in the inset of Figure 5a is the absorption from NI$^{\bullet-}$, measured at 400 nm, exclusively from self-quenching. Under these conditions (87% of the imide molecules are bound to DNA), it is clear that more NI$^{\bullet-}$ is produced when DNA is present. From the kinetic trace shown in Figure 5a, it is apparent that NI$^{\bullet-}$ decays on a significantly slower time scale (~1 ms). We have ruled out the possibility that the observed NI$^{\bullet-}$ is formed from bound NI-forming triplet states by estimating the absorbance change that would be observed if NI$^{\bullet-}$ were forming by this process. Using the extinction coefficient of NI$^{\bullet-}$, we estimate the

(22) We thank the reviewer for bringing this possibility to our attention.

maximum concentration of $NI^{\bullet-}$ formed in the presence of DNA that does not come from self-quenching in "bulk" aqueous solution is 0.5 μM. Given the concentration of NI not associated with DNA (5.5 μM), the self-quenching rate constant (1.4×10^9 M^{-1} s^{-1}), and the triplet state lifetime in the absence of quenching (162 μs), we estimate that, in the absence of any quenching by DNA, 56% of the triplet states are quenched by this process. With an estimate of the concentration of bound triplet states (2 μM), and the cage escape efficiency following self-quenching (0.11), we estimate that the maximum concentration of $NI^{\bullet-}$ that can be formed from self-quenching is 0.1 μM.

At lower concentrations of DNA, where the concentration of naphthalimide in the bulk aqueous phase is higher, $NI^{\bullet-}$ appears to be produced exclusively from self-quenching. A detailed analysis of the partitioning of $NI^{\bullet-}$ produced from self-quenching only (no added DNA) and from nucleotide oxidation has been carried out. From our study (results shown in Figure 2a of the Supporting Information), we conclude that when the concentration of nonassociated or "free" NI is less than ~10 μM, 50−100% (depending on the [NI]) of the total $NI^{\bullet-}$ observed by laser flash photolysis arises from electron transfer from DNA.[23] At higher concentrations (low ν (with DNA) or [NI] (in the absence of DNA)), self-quenching products are dominant and imide radical anion produced from DNA oxidation cannot be discerned.

In the range of DNA concentrations where self-quenching is minimized, the observed rate constants for $^3NI^*$ decay or $NI^{\bullet-}$ growth, measured at 480 and 400 nm, respectively, were, within experimental error, independent of the concentration of CT DNA base pairs added. Due to our limitations in the range of DNA concentrations that could be employed (at low DNA concentrations, self-quenching dominates), this is not surprising.

The identical experiments were carried with NDI (**2**). In the presence of DNA, a transient was observed immediately after pulsed excitation (Figure 5b). By comparison with Figure 2b, we assign this transient to the triplet state of the chromophore. This transient evolves to a species that has a spectrum identical to that of $NDI^{\bullet-}$ shown in Figure 3b. To assess the role of self-quenching in production of this radical, we systematically varied the concentration of nonassociated NDI by DNA addition. At each addition, the amount of $NDI^{\bullet-}$ produced from self-quenching was determined by pulsed excitation of an aqueous buffered solution of NDI only. The concentration of NDI in this solution corresponded to that in the bulk aqueous phase of the DNA-containing sample. Shown in the inset of Figure 5b, the yield of long-lived NDI radical is identical whether DNA is present. The concentration of NDI in the bulk aqueous phase was varied by the addition of DNA. The results of this study are shown in the Supporting Information. In all cases, the yield of $NDI^{\bullet-}$ with and without DNA were identical, suggesting that the yield of $NDI^{\bullet-}$ from redox interactions with DNA is negligible compared to that from self-quenching.

As additional amounts of DNA are added, the degree of imide/DNA complexation is increased. The yield of photoinduced transients was elucidated under varying degrees of complexation. For both imides, the magnitude of the nanosecond laser-induced changes associated with (a) ground-state bleaching at 340 and 380 nm (for NI and NDI, respectively) and (b) triplet-state absorbance at 480 and 485 nm (for NI and NDI, respectively) is significantly reduced as the degree of complexation increases. Under conditions where NI is predominantly bound, $^3NI^*$ is clearly observed as the sole transient immediately after the 8 ns laser pulse (Figure 5a). The spectrum evolves to that characteristic of the one-electron reduced NI. This result is initially surprising, given the fact that diffusion is not required prior to the electron transfer process. However, we have previously shown that $^3NI^*$ reacts primarily with isolated guanine nucleobases, and with a rate constant that is nearly 2 orders of magnitude slower than that expected for a diffusion-controlled reaction.[6] Thus, our observation of slow electron transfer within the NI/DNA complex is not surprising. Although the analogous experiment was carried out with NDI, it is apparent that $NDI^{\bullet-}$ produced comes predominantly from self-quenching processes in the bulk aqueous solution.

To investigate the role of the singlet excited state in nucleotide oxidation, fluorescence emission spectra were recorded as calf thymus DNA was titrated into pH 7.0 phosphate buffered solution (10 mM) of NI and NDI. In all cases, excitation was at the isosbestic point in the ground-state absorption spectrum (368 and 390 nm for NI and NDI, respectively). As calf thymus DNA was added to a buffered solution of NI, the fluorescence emission at 395 nm was diminished by a factor of 29 when 99% of the chromophrore was associated to the DNA. In an analogous experiment, the fluorescence intensity at 412 nm of NDI was also strongly quenched by a factor of 270 (99% of NDI bound to DNA) with the addition of DNA. In both cases, emission spectral shifts were not observed.

Discussion

Nucleotide Oxidation by Naphthalimide Excited States. The design of chemical systems as potential redox-active oligonucleotide cleavage agents must consider the reduction and oxidation potentials of the chromophore excited state and nucleotide units, respectively. Steenken and Jovanovic have determined the one-electron oxidation potentials of the G, A, T, and C nucleosides at pH 7 using pulse radiolytic techniques.[24] In order of decreasing ease of oxidation, the reduction potentials of the nucleoside radicals were determined to be 1.29 (G) < 1.42 (A) < 1.6 (C) < 1.7 (T) (V vs NHE at pH 7). Moreover, recent calculations have shown that -GG- steps are more easily oxidized than single guanine residues, with the HOMO localized almost exclusively on the 5′-side of the step.[25] Using the oxidation potentials of the individual nucleosides as estimates in DNA polymers,[26] it can be concluded that nucleotide oxidation will be exergonic when initiated by excited states whose reduction potential is more positive than ~1.3−1.7 V vs NHE. Using the half-wave reduction potentials of **1** and **2** determined in this work (see above), along with the zero−zero excitation energies of the T_1 and S_1 states of these chromophores,[6] we estimate the potentials to be (a) 1.5 and 2.6 V (vs NHE in aqueous solution) for reduction of $^3NI^*$ and $^1NI^*$, respectively and (b) 1.9 and 3.1 V (vs NHE in aqueous solution) for reduction of $^3NDI^*$ and $^1NDI^*$, respectively. Thus, we anticipate that forward electron transfer from all of the nucleotide bases to the singlet excited state of NI and NDI will be exergonic by at least 1 eV. Likewise, forward electron transfer from some of the nucleotide bases to the triplet excited states of NI and NDI will be only slightly exergonic. Redox tunability offers the prospect

(23) This fraction refers to the fraction of $NI^{\bullet-}$ produced in the presence of DNA that is over and above that from self-quenching (see Supplemental Information Figure 2). It does not imply anything about the fraction of triplet states that are quenched.

(24) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617−618.

(25) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063−7068; Prat, F.; Houk, K. N.; Foote, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 845−846.

(26) Although the oxidation potentials for only the individual nucleosides are known, they remain undetermined in DNA polymers, where ion solvation is potentially reduced.

of targeting the initial photochemistry to specific bases or subsets of bases. However, site-specific cleavage requires that the thermal events leading to strand cleavage can compete effectively with other events, such as "hole-hopping," that can rapidly move the oxidative damage to a thermodynamic sink, such as guanine nucleobases. Several recent reports have demonstrated that oxidative damage does indeed occur at sites far removed from the site of initial electron transfer.[1,27−29] These reports support hole-hopping and/or long-distance interactions to damage duplex DNA at remote guanine sites.

From a comparison of the transient spectra of $NI^{\bullet-}$ in Figure 3 with the spectra measured upon laser flash excitation of NI in the presence of calf thymus DNA (Figure 5a), it is apparent that photoreduction of NI by the native DNA is occurring to facilitate nucleotide oxidation. Since the one-electron reduction potential of $^3NI^*$ is only slightly higher than the oxidation potential of the purine bases (G and A), the most likely oxidative target is expected to be guanine-rich regions. Thus, using naphthalene imide triplet states, it will be difficult to discern between nucleobase-specific and hole-hopping mechanisms.

Singlet- vs Triplet-State Electron Transfer. The role of excited singlet and triplet states of the naphthalene imides in DNA oxidation was elucidated using a combination of absorption, fluorescence, and transient absorption experiments. The observation of significant fluorescence quenching as DNA is titrated into aqueous buffered solutions of NI or NDI suggests the availability of an efficient singlet-state quenching pathway in the presence of DNA. From picosecond pump−probe kinetic measurements, the lifetimes of the $^1NI^*$ and $^1NDI^*$ states have been determined to be 2.4 ns and 280 ps, respectively.[30] Thus, at the DNA concentrations employed (up to 800 μM and 180 μM nucleotide base pairs for NI and NDI, respectively), dynamic singlet-state quenching cannot be responsible for the observed fluorescence quenching. Thus, we attribute the strongly diminished fluorescence intensity to rapid electron transfer from the DNA nucleotides to the singlet excited state of NI or NDI within the preformed imide/DNA ground-state complex. Energy transfer from the singlet excited states of NI or NDI is not likely, since their excited singlet-state energies (3.4 and 3.2 eV, respectively[6]) are ~1 eV lower than those of the nucleotide bases.

As shown in Figure 5a, the formation of reduced NI is concomitant with triplet state decay. Although self-quenching does occur and also produces $NI^{\bullet-}$, it is clear that the amount of the radical species increases in the presence of DNA (Figure 5a inset). The results clearly suggest that nucleotide oxidation can be facilitated by the naphthalene imide triplet state. In the limited range of DNA concentrations (150−440 μM) where $NI^{\bullet-}$ is predominantly formed from nucleotide oxidation, the observed rate of radical production is, within experimental error, independent of DNA concentration. Our observation of the slow formation ($k_{obs} \approx 10^4$ s^{-1}) of $NI^{\bullet-}$ from the triplet precursor state does suggest that the process is a dynamic one resulting from reaction of nonassociated triplet states with the nucleotides. With the assumption that the process is dynamic, a bimolecular rate constant can be estimated from the observed rate constant of $NI^{\bullet-}$ formation ($k_{obs} = 10^4$ s^{-1}), the known triplet state lifetime ($k_d = 6.2 \times 10^3$ s^{-1}), and the nucleotide concentration employed in Figure 5a (202 μM). We estimate this rate constant to be $\sim 2 \times 10^7$ M^{-1} s^{-1}.[31] Saito et al. have reported the bimolecular quenching of the triplet state of an L-lysine derivative of 1,8-naphthalimide by duplex DNA.[5] The value estimated by us is consistent with the value of 5.3×10^7 M^{-1} s^{-1} reported by these workers. However, in our investigations, the kinetics cannot be studied over a widely varying range of nucleotide concentrations to conclusively show that the process is a diffusional one. From our previous kinetic studies with individual nucleotides,[6] it is clear that the NI triplet state undergoes electron transfer almost exclusively with guanine nucleotides since reactivity with the other nucleotides is more than 30 times slower. Moreover, the guanine reactivity is more than 2 orders of magnitude slower than that expected for a diffusion-controlled process. Since our data do not conclusively show that a dynamic process is occurring, the possibility of slow reactivity of a bound triplet state by a nearby guanine residue cannot be ruled out.

The roles of singlet excited states of the chromophores in nucleotide oxidation must also be considered when ground-state complexes are present. If significant concentrations of long-lived reduced imide or diimide were produced from rapid singlet-state quenching within the imide/DNA complex, it would be discernible in the post-nanosecond laser pulse spectrum. Under conditions where NI and NDI are predominantly associated with the native DNA, the laser-induced $T_1 - T_n$ absorption changes are substantially diminished (see below and Figure 6). The detailed relationship between singlet states that are "statically" quenched, triplet states that are formed, and reduced imide radicals produced is addressed below.

In attempts to time-resolve the reduced imide radical/oxidized nucleotide charge-separated state, picosecond pump−probe experiments were carried out. Aqueous buffered solutions of compounds **1** or **2** with calf thymus DNA were prepared where the imide or diimide was predominantly (>98%) bound to the DNA polymer. Immediately after 355 nm excitation with a 30 ps laser pulse, only trace amounts of imide or diimide radical anion were observed and persisted after 10 ns. Since charge-separated states were not observed in picosecond pump−probe experiments, forward charge transfer and recombination is occurring on time scales significantly shorter than 30 ps.

Relative Production of Triplet States with Added DNA. From (a) the decrease in apparent triplet quantum yield and (b) significant fluorescence quenching observed upon the addition of calf thymus DNA to an aqueous buffered solution of NI or NDI, we propose that rapid electron transfer, to $^1NI^*$ and $^1NDI^*$ from associated nucleotides of calf thymus DNA, is occurring. The ground-state equilibrium and excited-state interactions with calf thymus DNA are depicted in Scheme 1.

In Scheme 1, triplet excited states of the free imide (I) are produced upon excitation and intersystem crossing (pathway (a)). Near unity intersystem crossing yields (ϕ_{ISC}) have been previously reported for 1,8-naphthalimide and *N*,*N*-bis(2,2-dimethylpropyl)-1,4,5,8- naphthaldiimide in deoxygenated acetonitrile, but intersystem crossing of 1,8-naphthalimide is somewhat diminished in aqeuous solution due to hydrogen-bonding to solvent.[2,34] Using the $T_1 - T_n$ extinction coefficients of NI

(27) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1993**, *115*, 2437−2440.

(28) Meggers, E.; Michel-Beyerle, M. E.; Glese, B. *J. Am. Chem. Soc.* **1998**, *120*, 12950−12955.

(29) Barton, J. K. *Pure Appl. Chem.* **1998**, *70*, 873−879; Hall, D. B.; Kelley, S. O.; Barton, J. K. *Biochemistry* **1998**, *37*, 15933−15940; Nunez, M. E.; Hall, D. B.; Barton, J. K. *Chem. Biol.* **1999**, *6*, 85−97 and references therein.

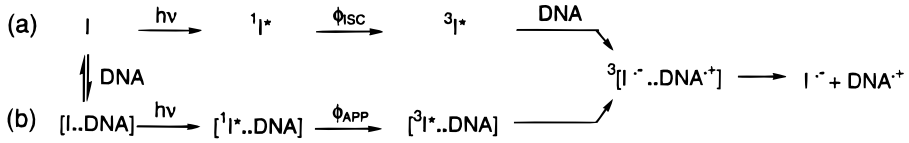
(30) Rogers and Kelly, unpublished data.

(31) Since the nucleotides in CT DNA are not homogeneously distributed, this bimolecular rate constant represents only an "average" bimolecular rate constant for quenching by the collection of nucleotides.

(32) (a) The concentration of imide excited states was determined by comparative actinometry using benzophenone in deaerated acetonitrile ($\phi_{ISC} = 1$ and $\epsilon_{T-T} = 6200$ M^{-1} cm^{-1} at 520 nm);[30b] (b) Bensasson, R.; Gramain, J. C. *J. Chem. Soc., Faraday Trans.* **1980**, *76*, 1801−1810.

(33) Brun, A. M.; Harriman, A. *J. Am. Chem. Soc.* **1991**, *113*, 8153−8159.

Scheme 1. Ground-State Association of the Imide (I) and Calf Thymus DNA[a]



[a] Pathways (a) and (b) show the formation of charge-separated states that are observed on the nanosecond laser flash photolysis time scale.

and NDI derivatives that were previously measured in 1:1 $CH_3CN:H_2O$,[6] we estimate ϕ_{ISC} to be 0.71 and 0.35 for NI and NDI, respectively. Of the "free" triplet states formed via pathway (a), a fraction of them will be diffusionally quenched to form solvated ion pairs, and some of the ion pairs will diffusionally separate, in competition with geminate recombination, to form $I^{\bullet-}$. We have previously determined that the efficiency of $I^{\bullet-}$ production following quenching of NI and NDI triplet states by the four individual nucleotides ranged from 19 to 37%, depending upon the imide and nucleotide employed.

At varying concentrations of calf thymus DNA, a fraction of the imide chromophores becomes associated with the DNA polymer and singlet-state quenching within the complex may occur. Since this process is in competition with intersystem crossing within the excited-state manifold of DNA-bound imide, the apparent intersystem crossing yield (ϕ_{APP}) is reduced. Moreover, in the case of both NI and NDI, the earliest resolvable transient (>30 ps) has spectral features characteristic of triplet-state only, indicating that radical products from singlet state quenching are not significant. From Scheme 1, the total triplet-state yield, observed from free and associated imide is given as ($\phi_{ISC} + \phi_{APP}$), the sum of that from pathway (a) and (b). Accordingly, the total $T_1 - T_n$ absorption change (ΔA_{T1}), observed immediately after the 8 ns laser pulse, is given by eq 4

$$\Delta A_{T1} = \Delta\epsilon_{T1}[^1I^*]_{TOT}\{\phi_{ISC}f_1 + \phi_{APP}(1 - f_1)\} = \Delta\epsilon_{T1}[^1I^*]_{TOT}\{(\phi_{ISC} - \phi_{APP})f_1 + \phi_{APP}\} \quad (4)$$

where, $\Delta\epsilon_{T1}$ is the $T_1 - T_n$ extinction coefficient, $[^1I^*]_{TOT}$ is the total concentration of electronically excited states produced, and f_1 is the fraction of imide molecules that are not associated with the calf thymus DNA (e.g., "free" imide). Assuming that the $T_1 - T_n$ extinction coefficients of "free" and "associated" imide are the same, and that the total number of excited states produced stays constant, eq 4 predicts that the laser-induced $T_1 - T_n$ absorption change should decrease linearly with decreasing f_1.The $T_1 - T_n$ absorbance changes, extrapolated to time zero, as a function of the fraction of "free" imide (f_1), are shown in a and b of Figure 6 for NI and NDI, respectively. Data have been corrected for the change in ground-state absorbance at 355 nm induced by binding to the calf thymus DNA. As predicted from eq 3, both plots are linear. Extrapolation of the data to $f_1 = 0$ yields the "limiting" intersystem crossing (ϕ_{APP}) of the imide while associated with calf thymus DNA. The $T_1 - T_n$ extinction coefficients ($\Delta\epsilon_{T1}$) have been previously determined for NI and NDI derivatives.[35] The concentration of excited states ($[^1I^*]_{TOT}$) was determined using a benzophenone actinometer solution. Using these values, along with linear least-squares analysis of the data shown in a and b of Figure 6, we estimate ϕ_{APP} to be 0.08 ± 0.02 and 0.004 ± 0.02 for NI and NDI. The results are consistent with the clearly nonzero intercept in Figure 6a vs 6b.[36] The large error on the latter value is a a result of the large uncertainty in the near-zero intercept in Figure 6b. Correspondingly, the ϕ_{ISC} values derived from this treatment (ϕ_{ISC} = 0.79 ± 0.06 and 0.41 ± 0.05 for NI and NDI, respectively) are in good agreement with those estimated from pulsed photolysis of aqueous solutions of NI and NDI in the absence of DNA. The fraction of triplet states that is produced in competition with rapid singlet state quenching (ϕ_{APP}) is significantly larger in the case of NI than NDI.

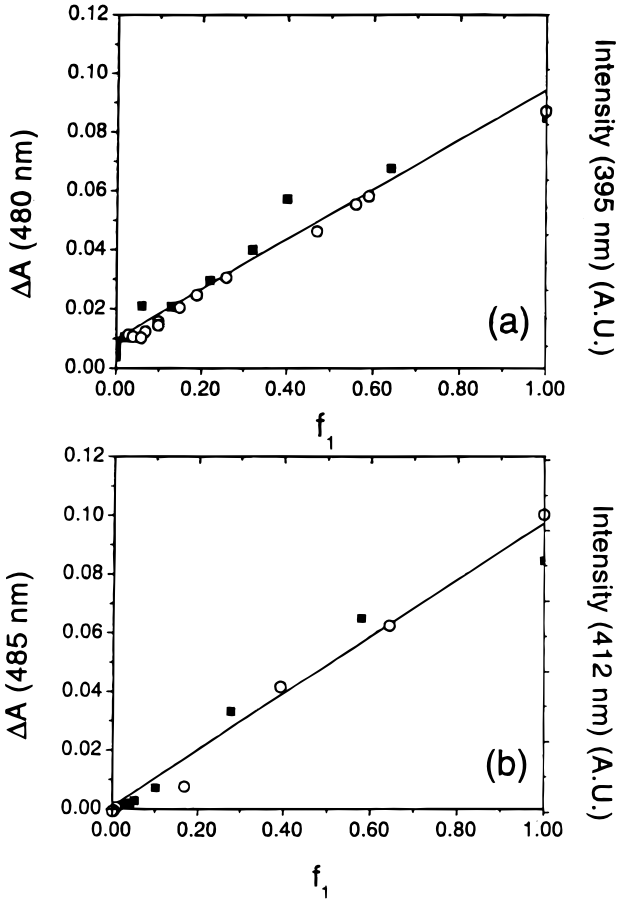


Figure 6. $T_1 - T_n$ absorbance changes ((■) extrapolated to time = 0 after the laser pulse) induced upon 355 nm excitation of a solution of (a) NI and (b) NDI at 480 and 485 nm, respectively, as a function of the fraction of ground states that are not associated (f_1 = fraction of "free" imides) with calf thymus DNA. All solutions are prepared in deoxygenated phosphate buffer solution (10 mM, pH 7.0). Data have been normalized for changes in ground-state absorbance at 355 nm upon complexation. Superimposed on these graphs are measured fluorescence intensities (○) at (a) 395 nm and (b) 412 nm for NI and NDI as calf thymus DNA was added. In both (a) and (b) fluorescence excitation was at the isosbestic point in the ground-state spectrum.

From the intersystem crossing quantum yields of NI and NDI, along with the measured singlet-state lifetimes ($\tau_{S1} = 1/k_{S1} =$

(34) Samata, A.; Ramacharndram, B.; Saroja, G. J. *Photochem. Photobiol., A* **1996**, *101*, 29−32.

(35) The determinations of ϕ_{APP} in aqueous solution were carried out using the assumption that the triplet absorption coefficients of NI and NDI were identical to those reported earlier (ref 6) in 50:50 $CH_3CN:H_2O$.

(36) The apparent "scatter" in the absorption changes corresponding to small f_1 values is a consequence of the small transient absorption signals in this range. Thus, the analysis in Figure 6, where the ISC efficiency is extrapolated to fully bound conditions, provides a more accurate determination of ϕ_{APP}.

2.4 ns (NI) and 280 ps (NDI)), the rate constants for intersystem crossing (k_{ISC}) are 3.3×10^8 s^{-1} and 1.5×10^9 s^{-1} for NI and NDI, respectively. In both cases, the decrease in ISC quantum yield suggests a competing pathway for S_1 deactivation when the chromophores are bound to the DNA. The rate constants (k_{et}) for this competitive pathway are evaluated from eq 5.

$$\frac{\phi_{ISC}}{\phi_{APP}} = 1 + \frac{k_{et}}{k_{S1}} \quad (5)$$

From eq 5 and the quantum yields given above, we estimate the rate constants for this competive process to be 3.7 ($\pm$ 0.07) $\times 10^9$ s^{-1} and 3.6 ($\pm$ 15) $\times 10^{11}$ s^{-1} for reaction of the imide singlet states with DNA.[37] Assuming that the competitive process is electron transfer, it is clear that, in both cases, electron transfer competes very effectively with intersystem crossing. Although the rate constant for intersystem crossing in NDI is higher than that of NI, the lower apparent triplet yield of the former can be attributed to the more rapid photoinduced electron transfer to the singlet state of NDI. This result is consistent with NDI being a better electron acceptor than NI.

Our observation of fluorescence quenching with added DNA is consistent with Scheme 1. Owing to the rapid singlet-state quenching within the imide/DNA complex, the fluorescence intensity is significantly diminished. Consistent with Scheme 1, fluorescence is predominantly observed only from electronically excited imide singlet states that are free in solution. Thus, it is anticipated that the fluorescence intensity will increase linearly with f_1. These data are superimposed in a and b of Figure 6 for NI and NDI, respectively. The linear dependence is consistent with the model shown in Scheme 1.

The issue of competitive electron transfer and intersystem crossing for intercalated photocleavage agents has been addressed for other compounds. In related experiments, Brun and Harriman investigated the photochemistry of intercalated quaternary diaazaaromatic salts.[38] Consistent with the observations reported herein, these workers observed significant fluorescence quenching ($\phi_{f^0}/\phi_f \approx 20$ or 50, depending upon the chromophore employed) upon intercalation of the dye. The reduced chromophore was observed by picosecond pump−probe spectroscopy. First-order charge recombination occurred rapidly (τ_{rec} < 100 ps) but apparently on a somewhat slower time scale than singlet ion-pair recombination in our work. In other work, singlet vs triplet ion pairs have been considered in the cleavage of duplex DNA by intercalated anthraquinone.[39] It was postulated that efficient cleavage of DNA does not occur from singlet-state precursors, since rapid charge recombination occurs before strand cleavage and hole hopping takes place.

Significance to Efficient DNA Photocleavage. One report has addressed oligonucleotide cleavage resulting from irradiation of an L-lysine derivative of 1,8-naphthalimide.[5] In the former case, photocleavage of a duplex hexamer was observed under conditions of steady-state irradiation. Using laser flash, the reduced naphthalimide was observed as a product of the bimolecular quenching of the triplet excited state. Ground-state association by the planar 1,8-naphthalimide with the oligonucleotide was not addressed, and the relative yield of redox products, as a function of complexation, was not determined. Moreover, the ground-state concentrations used by these workers are sufficiently high (100 μM) that radical anions from self-quenching could certainly arise. From our studies (see above), significant concentrations of NI$^{\bullet-}$ were produced at concentrations that were an order of magnitude smaller. In published reports of DNA oxidation and cleavage using naphthalene imide chromophores,[4,5] this mechanism was not discussed.

In a second report, strand scission of supercoiled DNA in the presence of bis(substituted)-1,4,5,8-naphthaldiimide was investigated.[3] A bis(hydroperoxy) derivative of the naphthaldiimide was shown to spontaneously nick supercoiled DNA. However, in control experiments, naphthaldiimide derivatives that lacked the pendant hydroperoxide substituent did not cleave the DNA, even after piperidine treatment. The result is perplexing, considering the fact that derivatives of 1,4,5,8-naphthalimides are more powerful oxidizing agents in their electronically excited singlet and triplet states. Since, on time scales slower than 30 ps, singlet-derived products are not observed with either compound, charge recombination is apparently rapid. Under conditions where NDI derivatives are predominantly associated with the DNA and such "static" processes can occur, the photocleavage efficiency will be severely diminished. Thus, it is critical to consider the partitioning of "free" and "bound" excited states when correlating results from separate studies. From our observation of the data presented herein, the contrasting photoreactivities of naphthalimide and naphthaldiimide may be a result of differing degrees of association (and thus differing degrees of static quenching processes) within a ground-state complex.

From our systematic correlation of the relative yield of long-lived naphthalimide and naphthaldiimide radical with the fraction of bound chromophore, it is apparent that the comparison of photocleavage quantum yields (and yields of the radical intermediates) must consider the relative role of singlet- and triplet-state electron transfer. As the data in Figure 6 clearly show, rapid singlet-state quenching within the imide/DNA complex results in diminished product yield. In the case of compound **2**, extrapolation of the transient absorption data to the "fully bound" conditions leads to insignificant product formation, suggesting that rapid charge-recombination, within the complex, is occurring. In contrast, intersystem crossing in compound **1**, even when predominantly bound, is apparently competitive with singlet-state quenching. This is evidenced by the nonzero intercept in Figure 6a. The finite amounts of reduced NI formed as a result of competitive intersystem crossing is likely a result of slower charge recombination within the triplet vs singlet radical ion pair.

Conclusions

In summary, we have carried out detailed investigations to characterize the photophysical and photochemical properties of naphthalene imide and diimide derivatives in the presence of DNA. The systematic investigations correlate the extent of chromophore/DNA complexation with triplet-state and radical production following pulsed photolysis. Significantly, the relative yield of long-lived triplet excited states produced decreases linearly as the fraction of associated chromophores increases. In the case of the 1,8-naphthalimide derivative (**1**) employed in this work, the intersystem crossing efficiency within the ground-state complex is 0.08. In contrast, intersystem crossing within the ground-state complex of NDI and calf thymus DNA is negligible (0.004), and only trace amounts of radical products

(37) The electron-transfer rate constants from the intersystem crossing efficiencies (eq 5) were estimated assuming that $T_1 - T_n$ extinction coefficients of "free" and "bound" imides are identical.

(38) Brun, A. M.; Harriman, A. *J. Am. Chem. Soc.* **1991**, *113*, 8153−8159.

(39) Ly, D.; Kan, Y.; Armitage, B.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 8747−8748.

are observed. The design of chemical systems that can initiate site-specific damage in biological polymers requires efficient and selective association with the target of interest. Hence, it is critical to understand and circumvent the factors responsible for "short-circuiting" the photochemical pathway. Strategies for increasing the intersystem crossing efficiency and spatial charge separation are currently being explored.

For both compounds employed, radical anion production from self-quenching processes is significant. In the case of NI, additional radical anion is produced when DNA is present, suggesting that nucleotide oxidation is occurring. Conversely, when NDI is used, all of the NDI radical anion that is observed by transient absorption can be accounted for by self-quenching interactions. The observation that additional $NDI^{\bullet-}$ is not produced from DNA-bound molecules is consistent with very rapid electron transfer and charge recombination processes initiated by the electronically excited singlet state.

Acknowledgment. We thank the American Cancer Society, Maryland Division, for financial support. J.E.R. and S.J.W. are grateful for Research Assistantship support from the UMBC Graduate School (DRIF).

Supporting Information Available: Transient absorption data fit to eq 3. Production of radical anion from self-quenching and DNA oxidation as a function of imide and diimide concentrations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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