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Excited-State Lifetimes in a G-C DNA Duplex are nearly Independent of Helix Conformation and Base Pairing Motif

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DNA excited-state dynamics; DNA charge transport; exciplex; nonradiative decay

Ultraviolet (UV) sunlight is absorbed strongly by the DNA nucleobases, creating excited electronic states, which can initiate photoreactions that result in mutations and genomic instability. Time-resolved absorption[1,2] and emission experiments[3–5] have shown that nonradiative deactivation of excited states of single nucleobases occurs primarily on a femtosecond timescale. Ultrafast decay to the electronic ground state is an attribute of highly photostable compounds,[6] and it has been proposed more than once that DNA photodamage may be reduced because its molecular building blocks share the high resistance to UV photodamage of absorptive sunscreens.[7,8] However, the suggestion that photophysical properties of single bases carry over to polymeric DNA is undercut by reports, both old and new, of emissive excited states with nanosecond lifetimes.[9–13] Recent femtosecond experiments have now confirmed that excited states of DNA base multimers relax by fundamentally different pathways.[14] Nanosecond lifetimes are rare and instead long-lived ‘dark’ excited states with characteristic lifetimes of up to 100 ps are commonplace in single- and double-stranded oligonucleotides,[15] polynucleotides,[16,17] and even dinucleotides.[18]

These long-lived states are proposed to be charge-transfer states or exciplexes formed when an electron is partially transferred between π -stacked nucleobases.[15,18] The picosecond lifetimes of these states suggest that they decay rapidly by charge recombination. In support of this interpretation, it was recently shown that exciplex lifetimes in a series of dinucleotides depend sensitively on the thermodynamic driving force for back-electron transfer.[18] Motivated by evidence that the conformation of the base stack dramatically affects rates of excess charge transport in DNA,[19] we chose to investigate how base step geometry affects excited-state dynamics. Here, we report the lifetimes of singlet excited states for a duplex of constant base sequence that can be switched into various helical conformations comprised of both Watson-Crick (WC) and Hoogsteen base pairs. Surprisingly, the lifetimes of the long-lived or exciplex states are nearly unchanged even when the base-stacking geometry and base-pairing motif are strongly altered.

Transient absorption signals recorded at a pump wavelength of 266 nm and probe wavelengths of 250 nm and 570 nm are compared in Figure 1 for the duplex d(GC)₉-d(GC)₉ in B- and Z-helix conformations. The Z-form structure was generated at high ionic strength and confirmed by UV absorption and CD spectra (Figure 2). Also shown are signals recorded for an equimolar mixture of the RNA mononucleotides guanosine 5'-monophosphate (GMP) and cytidine 5'-monophosphate (CMP).

In Figure 1, the induced absorbance change (ΔA) is negative at 250 nm due to bleaching of the ground-state population.[18,20] The time needed for the 250 nm signals to recover to baseline measures the time required to repopulate the ground state. On the other hand, the signals at 570 nm monitor excited-state population. The slower decay of the mononucleotide signals at 250 nm compared to 570 nm is due to rate-limiting vibrational cooling at the former wavelength.[15,21] In the equimolar mononucleotide mixture, the 570 nm signal is well described by a single exponential decay with a time constant of 0.6 ± 0.1 ps. At 250 nm, the signal from the mononucleotide mixture decays biexponentially with a major component of 2.0 ± 0.3 ps and a much weaker one with a lifetime of ~ 30 ps. The ~ 2 ps decay component is assigned to vibrational cooling following ultrafast internal conversion to the electronic ground state.[22] The weak ~ 30 ps component is assigned to the $^1n\pi^*$ state of cytosine, which is populated via decay from the initial $^1\pi\pi^*$ state.[22]

The signals from the d(GC)₉-d(GC)₉ structures decay significantly more slowly than ones from the equimolar mixture of mononucleotides, as we reported previously for the B-form helix.[20] The oligonucleotide signals are well fit by single exponential decays at 250 and 570 nm. A constant offset was used to fit the transients at 250 nm, which exhibit weak residual bleaches at long times, possibly due to unidentified photoproducts formed in low yields. The addition of a second exponential term did not improve the quality of fits for any of the bleach recovery signals. Fitting parameters are summarized in Table 1.

The decay times at 250 nm and 570 nm are more similar for the oligonucleotides than for the equimolar mixture of GMP and CMP (Figure 1 and Table 1). Previously, a ~ 2 ps decay was observed at 250 nm in AT-oligonucleotides and assigned to vibrational cooling following subpicosecond internal conversion by excited states localized on unstacked bases.[15] There is no evidence of a ~ 2 ps decay in the bleach recovery signals of the B- and Z-form duplexes. The absence of a ~ 2 ps decay component at 250 nm and the lack of a subpicosecond lifetime at 570 nm suggests that there is very little subpicosecond internal conversion to the electronic ground state in the d(GC)₉-d(GC)₉ systems. This is consistent with the greater degree of base stacking and higher melting temperature of this GC-rich sequence. The approximately 50% slower decays observed at 250 nm compared to 570 nm in the case of B- and Z-forms of d(GC)₉-d(GC)₉ are likely due to vibrational cooling following decay of the relatively short-lived excited states probed at 570 nm.

The signal at 570 nm is free from ground-state vibrational-cooling dynamics. At this probe wavelength, the decay time increases by nearly an order of magnitude for the B- and Z-forms compared to the equimolar mononucleotide mixture. This striking finding contrasts dramatically with results from time-resolved emission experiments. Fluorescence upconversion signals from the related duplex poly(dGdC)-poly(dGdC) actually decay more rapidly than ones from the dG and dC mononucleotides.[23] This is evidence that the signal decay at 570 nm is due to dark states, which we have assigned to exciplexes.[20]

The main finding of this study is the similar excited-state dynamics for B- and Z-form structures in H₂O solution (Figure 1b). The decay times for the Z-form are only $\sim 20\%$ slower than for the B-form at both probe wavelengths (Table 1). George and coworkers studied transient changes to the vibrational spectra of B- and Z-forms of the closely related

homoduplex of poly(dGdC) in D₂O using the ps-TRIR technique with UV excitation at 267 nm.[24, 25] They reported that vibrational bleach signals of the B-form duplex decay biexponentially, while the Z-form bleach signals exhibit monoexponential dynamics. Despite this reported difference, inspection of their decay curves suggests that the overall decays for the B- and Z-forms are similar, in agreement with our results.

Interestingly, the IR bleach signals reported by George and coworkers decay more slowly than our UV bleach signals (compare Figures S2 and S3 of ref. [25] with Figure 1b). This is due to the different solvent conditions: our experiments were carried out in H₂O, while those of George and coworkers were carried out in D₂O. We have confirmed that the UV bleach signals of B-form d(GC)₉-d(GC)₉ in D₂O relax more slowly than in H₂O with the appearance of a long-lived decay component of ~30 ps. This remarkable isotope effect will be discussed in a future publication. A deuterium kinetic isotope effect was reported earlier for alternating AT-oligonucleotides.[15]

Given discussions that long-lived excited states in DNA base multimers are exciplexes that decay by charge recombination,[15,18] it is important to consider how helix conformation might affect exciplex lifetimes. We initially expected exciplex lifetimes to differ in B- and Z-DNA given the different base-base overlaps in these structures (Figure 3a). Rates of charge transfer reactions are frequently sensitive to donor-acceptor orientation due to the effect of orientation on electronic coupling. Note that although the geometries in Figure 3a are appropriate for the ground state, we do not expect large geometrical changes in the excited state given its short lifetime.

Charge transfer reactions in DNA are known experimentally to be sensitive to conformation,[19] and it was predicted theoretically that charge migration rates could vary several hundred-fold as the duplex conformation is altered.[26] Theoretical studies have shown order of magnitude changes in the electronic coupling responsible for hole transfer (HT) or excess electron transfer (EET) in DNA with subtle changes in conformation.[26–29] Nonetheless, important differences exist between the electronic coupling for HT or EET and the coupling that describes exciplex decay. HT and EET in DNA are charge-shift reactions involving a nucleobase radical ion and a neutral base. Studies of HT or EET along a DNA strand usually involve a donor and acceptor separated by at least one intervening base pair. In this case, electron transfer is thought to occur in the nonadiabatic limit in which the rate varies as the square of the electronic coupling. On the other hand, the decay of an exciplex with a high degree of charge-transfer character involves charge recombination by oppositely charged radical ions on adjacent base pairs. Both HT and EET occur with a free energy change of nearly zero, but exciplex decay is strongly exoergic. The much greater energy splitting between adiabatic states in the case of an exciplex will increase the electronic coupling for charge recombination compared to HT or EET.

We propose that the electronic coupling relevant to exciplex decay is large and relatively insensitive to the geometry of the stacked base pairs. The coupling is likely large enough that a nonadiabatic electron transfer description is simply inappropriate for describing charge recombination by DNA exciplex states. Although nonadiabatic theory has been used to describe charge recombination in some contact ion pairs,[30] this approach may fail for exciplexes having only partial electron transfer character.[31,32]

Excited-state lifetimes in dinucleotides have been shown to decrease with decreasing energy of the exciplex state formed by interbase electron transfer.[18] How might the energy of a GC exciplex change with helix conformation? Sugiyama et al. studied the effect of helix conformation on the ionization energy of GG/CC base pairs.[33] The results showed only an ~8% change in ionization potentials among the A, B, and Z conformations.[33] If the

electron affinity also changes only weakly with helix conformation, then the driving force for back electron transfer remains approximately the same. In this case, the exciplex lifetimes, which are otherwise highly sensitive to ΔG , [18] remain the same for different helix conformations.

Finally, although there are great differences in how the G-C base pairs are arranged in the B- and Z-form duplexes (Figure 3a), both are made up of identical WC base pairs. To rule out the possibility that the similar signals are due to photoprocesses occurring within WC base pairs, [34–36] measurements were performed on the ‘Hoogsteen duplex’ formed from d(GC)₉-d(GC)₉ at low pH (Figure 4). Even though its precise conformation is uncertain, there is clear evidence that this duplex has Hoogsteen base pairing (Figure 3b) under our experimental conditions. [37] The pronounced structural change induced by lowering the pH is also manifested in the UV absorption and CD spectra (Figure 2).

Essentially identical signals were observed in the B-form duplex with WC base pairs at pH 6.8 and the Hoogsteen duplex at reduced pH (Figure 4). It has been suggested that ultrafast nonradiative decay via electron-coupled proton transfer takes place in the WC base pair, but not in non-WC base pairs. [35,38] Here, there is little difference in dynamics for these very different base-pairing motifs, suggesting that the gas-phase decay channel does not affect the long-time dynamics of these base-stacked DNAs. The nearly identical signals for these d(GC)₉-d(GC)₉ structures, combined with a previous study on poly(A) and poly(dA) [16,17] that showed identical long-time signals despite their structural differences, support the conclusion that long-lived excited states in both single- and double-stranded systems have lifetimes that are approximately the same for different helix conformations having the same base sequence.

We have framed our discussion in terms of the expected dynamics of exciplex states with significant charge transfer character. In an alternative model, the long-lived signals are assigned to dynamics of delocalized excitons formed from the $^1\pi\pi^*$ states. [39] However, there is no basis for expecting the excitonic states to be independent of helix conformation. In fact, computational studies establish the opposite conclusion. [40] It is worth noting that differences in the UV absorption and CD spectra of the various duplexes (Figure 2) arise because of differences in the excitonic states. Given these differences, it is difficult to explain how excitons could give rise to nearly identical dynamics in all systems. Instead, the common decay times reported here for DNAs that differ in their sets of excitonic states are best explained by the dynamics of localized excited states or exciplexes, which decay at rates that are independent of helix conformation for the reasons described above.

In summary, excited-state lifetimes in the DNA duplex d(GC)₉-d(GC)₉ are largely independent of helix conformation. Exciplex decay in DNA is proposed to depend strongly on ΔG , but weakly or not at all on the electronic coupling for charge recombination, even though the latter may nonetheless be quite large. The photostability of the double helix may be assured by rapid electron-hole recombination, which occurs at uniformly high rates in all helix conformations. Theoretical studies of charge recombination in short-distance electron-hole pairs in DNA will be essential for a definitive explanation. Such work will deepen the emerging contacts between the fields of DNA charge transport and DNA photophysics.

Experimental Section

Transient absorption signals were recorded using an amplified titanium-sapphire laser system, as described elsewhere in more detail. [41] Pump pulses, with a center wavelength of 266 nm, were obtained by harmonic generation. UV probe pulses at 250 nm were generated using an optical parametric amplifier, and visible probe pulses were produced by white light continuum generation in a 1 cm path length cell. Polarization of the pump pulse relative to

the probe pulse was set at magic angle (54.7°). Spectral isolation of the UV probe pulse was accomplished after the sample using a Jobin Yvon (Edison, NJ, USA) H10-D-UV monochromator with 0.5–1 mm slits, and signals were detected with a photomultiplier tube. Wavelength selection for visible probe pulses was carried out with a 10 nm interference filter placed after the sample, and the signal was detected using an amplified Si photodiode. Pump pulse intensities were typically in the range of 0.2–2 GW cm⁻² and the pump pulse diameter was three to five times larger than that of the probe pulse. Transient absorption measurements were conducted on samples held in a 1.2 mm path length spin cell with calcium fluoride windows rotated at several hundred revolutions per minute. Samples were monitored for photodegradation by UV/Vis absorption spectroscopy during the course of the experiment. In general, a change of less than 2–5% was observed at the maximum absorbance wavelength during the course of the measurements. Transient decays were globally fit using the IGOR Pro 5.04 software to sums of exponentials analytically convoluted with a Gaussian instrument response of 250 fs. The first ~300–700 fs of data after time zero was excluded from the fits due to interfering signals from the solvent and the sample cell.

Guanine and cytidine 5'-monophosphates were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) and dissolved in H₂O containing 25 mM phosphate buffer (pH 6.8) and 0.25 M NaCl. The oligonucleotide d(GC)₉ was obtained from Midland Certified Reagent Company (Midland, TX, USA) as a lyophilized powder. The B-form duplex was prepared by dissolving d(GC)₉ in 25 mM aqueous phosphate buffer containing 0.25 M NaCl. To form the Z-conformation, d(GC)₉ was dissolved in 25 mM aqueous phosphate buffer containing 5.0 M NaCl. The Hoogsteen base-paired duplex, d(GC)₉ was formed by adding dilute HCl dropwise to deionized water containing 0.25 M NaCl until pH 3.6 was reached. All duplexes were thermally annealed by heating to 70°C, followed by slow cooling to room temperature. Solutions were prepared to have an absorbance of ~1 at the pump wavelength of 266 nm.

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Figure 1.

Normalized transient absorption signals in buffered H₂O solution at probe wavelengths of 250 nm and 570 nm for a) an equimolar mixture of the guanine and cytosine mononucleotides and for b) d(GC)₉-d(GC)₉ in B-form vs. Z-form. Solid lines are best-fit curves using the parameters in Table 1. Signals at 570 nm were corrected for two-photon ionization of the solvent by the pump pulse as described previously. [16,17]

Figure 2.

a) a) Absorption and b) circular dichroism spectra for the B- (solid curve), Z-, (dashed curve), and Hoogsteen (dotted curve) conformations of $d(GC)_9 \cdot d(GC)_9$.

Figure 3.
a) Base-base overlaps for CpG and GpC steps of B- and Z-conformations viewed along the helix axis. b) Watson-Crick and Hoogsteen G-C base pairing motifs.

Figure 4. Normalized transient absorption signals (266 nm pump/250 nm probe) for d(GC)₉-d(GC)₉ in B-form and Hoogsteen duplex structures.

Table 1

Best-fit parameters for transient absorption signals for d(GC)₉-d(GC)₉ in the indicated helix conformations and excited at 266 nm.^[a]

Sample	Probe Wavelength	A ₁ (%)	τ (ps)	A ₂ (%)
B-form ^[b]	250 nm	98 ± 4	6.3 ± 0.6	2 ± 2
	570 nm	100	4.1 ± 0.3	---
Z-form ^[c]	250 nm	98 ± 4	7.6 ± 0.8	2 ± 2
	570 nm	100	4.8 ± 0.4	---
Hoogsteen ^[d]	250 nm	96 ± 4	5.3 ± 0.6	4 ± 1

^[a] The fitting function, $A_1 \exp(-t/\tau) + A_2$, was convoluted with a Gaussian function with a full-width at half maximum of 250 fs to simulate the instrument response function. All uncertainties are twice the standard error.

^[b] pH 6.8, 0.25 M NaCl

^[c] pH 6.8, 5 M NaCl

^[d] pH 3.6, 0.25 M NaCl