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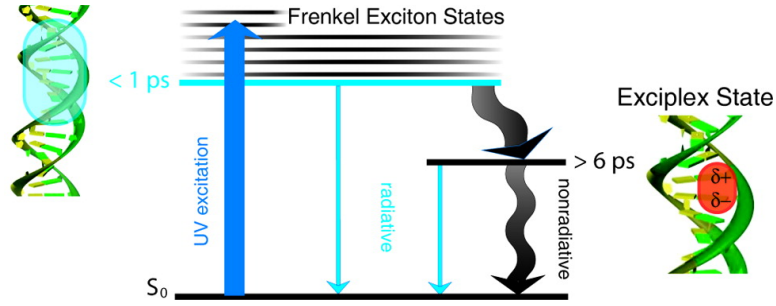

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Ground-State Recovery Following UV Excitation is Much Slower in G·C−DNA Duplexes and Hairpins Than in Mononucleotides

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There is keen interest in excited electronic states created in DNA by the absorption of UV light. Knowledge of the relaxation pathways for excess electronic energy is vital for understanding the complex dependence of DNA photodamage on sequence and secondary structure. Attention has recently been focused on base stacking and base pairing, the twin architectural motifs of the DNA double helix, and the role they play in mediating the decay of excess electronic energy.[1–4] Studies of how hydrogen bonding and π-stacking affect photoprocesses in DNA may also provide new insights into analogous interactions in photonic polymers made of aromatic chromophores.

Transient absorption measurements by our group[2,5] and by others[6,7] have established that excited states in DNA polymers and oligomers containing the bases A and T relax orders of magnitude more slowly than excited states of A or T alone. The existence of long-lived excited states in DNA model systems with G·C base pairs is uncertain, but extremely topical in light of recent discussions of possible ultrafast quenching channels.[8–11] Here, we report a femtosecond transient absorption study of excited-state relaxation in double-stranded oligonucleotides containing G·C base pairs. Bleach recovery signals indicate that excess electronic energy relaxes about an order of magnitude more slowly in all systems studied than in the single mononucleotides of G and C.

Transient absorption signals recorded with excitation at 267 nm and probing at 250 and 570 nm are shown for a variety of oligonucleotides made of G·C base pairs in Figure 1. The signals recorded for an equimolar mixture of guanosine 5′-monophosphate (GMP) and cytidine 5′-monophosphate (CMP) is also shown for comparison. The positive signals from the oligomers at 570 nm are assigned to dark excited states that do no contribute significantly to the emission (see below). Negative signals are seen for all systems at 250 nm owing to depopulation of the electronic ground state by the pump pulse. The signals show approximate mirror-image symmetry with the exception of the CMP and GMP mixture due to rate-limiting vibrational cooling ($\tau \approx 2$ ps) at 250 nm.[12] Recovery of the 250 nm signals to baseline directly measures the time for molecules excited by the UV pump pulse to return to the electronic ground state. This recovery is fastest for the equimolar mixture of the 5′-mononucleotides GMP and CMP. Slower, approximately single exponential recovery is seen for the $d(GC)_9$·$d(GC)_9$ duplex with its stacks of alternating G·C base pairs. Even slower, biexponential decays are seen in the self-complementary octamer $d(C_4G_4)$, and in the hairpin-forming oligonucleotides $d(C_5T_4G_5)$ and $d(C_5A_4G_5)$. The latter three systems have mainly G-on-G and C-on-C base stacking. The good agreement of the transients from the two hairpins suggests that these signals arise from stacked base pairs in their common G·C stem regions and not from poorly stacked bases in the hairpin loops.[13]

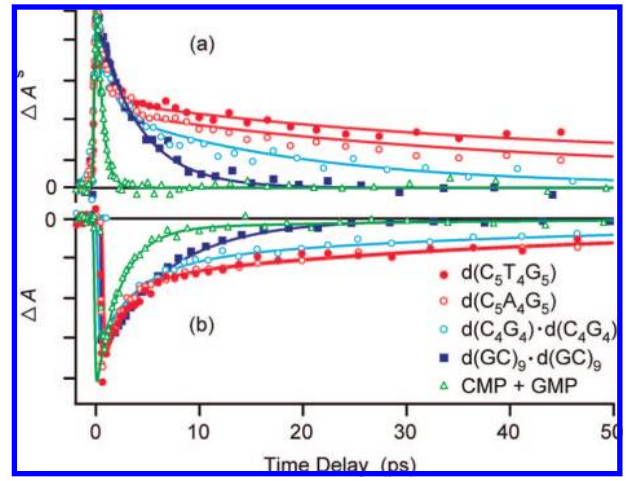

Figure 1. Normalized femtosecond transient absorption signals excited at 267 nm for various G·C duplexes and hairpins and an equimolar mixture of CMP and GMP and probed at (a) 570 nm (corrected for two-photon ionization of the solvent), and (b) 250 nm. Best-fit curves are shown by solid lines.

Table 1. Time Constants From Global Fits to the Signals in Figure 1

system	probe wavelength (nm)	τ_1 (ps)	τ_2 (ps)
CMP + GMP	570	0.6 ± 0.1	
	250	2.0 ± 0.3	31 ± 26
$d(GC)_9$·$d(GC)_9$	570	4.1 ± 0.3	
	250	6.3 ± 0.6	
$d(C_4G_4)$·$d(C_4G_4)$	570	1.3 ± 0.3	22 ± 6
	250	3.3 ± 0.6	*a*
$d(C_5A_4G_5)$, $d(C_5T_4G_5)$	570	1.1 ± 0.2	41 ± 6
	250	3.0 ± 0.4	*a*

[a] Globally linked for the 250 and 570 nm transients.

Fits to the transients in Figure 1 (see Tables 1 and S1) indicate that recovery to the electronic ground state takes many picoseconds in GC-oligonucleotides following UV photoexcitation, even though relaxation by the mononucleotides of G and C occurs primarily on a subpicosecond time scale.[14,15] The very weak picosecond decay component seen in CMP + GMP arises from the $^1n\pi^*$ state of CMP.[15] Detection of long-lived excited states in G·C oligonucleotides extends our earlier observation of slow ground-state recovery in A·T systems.[2] It also contrasts dramatically with time-resolved emission studies. Emission from poly(dGdC)·poly(dGdC) was recently studied by Miannay et al. by the fluorescence upconversion technique.[16] These authors reported an average lifetime of 200 fs for the multiexponential emission decay at 330 nm. This value is about half the average lifetime of 450 and 340 fs seen for emission by the single DNA nucleotides dCMP and dGMP, respectively.[17] Although fluorescence from poly(dGdC)·poly(dGdC) decays more rapidly than in dCMP and dGMP, ground-state recovery in the

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related duplex $d(GC)_9 \cdot d(GC)_9$ occurs roughly an order of magnitude more slowly than in its constituent building blocks (Figure 1).

These apparently contradictory findings are easily reconciled by a model in which fluorescent excited states rapidly and irreversibly decay to dark excited states. The radiative decay rate of these states is too low for detection by femtosecond fluorescence up-conversion, yet they are readily seen by depletion of the ground-state population or excited-state absorption at visible wavelengths (Figure 1). The fluorescence lifetime characterizes the decay of a bright excited state, but generally only equals the time for ground-state recovery in a two-level system. This is a common reason for frequently observed differences between transient emission and transient absorption lifetimes in complex molecular systems.[18] Our results clearly indicate that the ultrafast lifetime observed by Miannay et al.[16] does not measure the time needed for excited states to reach the ground state.

The long-lived states are proposed to be exciplexes or excimers formed by partial charge transfer between two stacked bases.[2,19] This explains their dark character, and the rapid loss of fluorescence is evidence that they are populated on a subpicosecond time scale. The faster rate of decay of these states in the alternating $d(GC)_9 \cdot d(GC)_9$ duplex compared to the nonalternating systems parallels the behavior seen in A·T oligonucleotides.[2] Recently, it was shown that DNA exciplex lifetimes decrease with increasing thermodynamic driving force for charge recombination.[19] The shorter lifetime seen for the alternating $d(GC)_9 \cdot d(GC)_9$ duplex compared to the other systems is consistent with the lower energy of the G^+C^- vs the G^+G^- and C^+C^- ion pair states.

The slow decays in Figure 1 reveal that Watson−Crick G·C base pairs do not accelerate relaxation to the electronic ground-state compared to the individual bases. On the basis of ab initio calculations, Domcke and Sobolewski suggested that proton motion in a UV-excited Watson−Crick G·C base pair leads to ultrafast relaxation to the electronic ground state.[8,10] This fascinating proposal has been invoked to explain experiments on single G·C base pairs in isolation[9] and in nonpolar solution.[11] Schwalb et al.[11] reported that the fluorescence lifetime of a single base pair composed of G and C derivatives is 0.355 ps in chloroform compared to lifetimes of 0.67 ps for C and 0.84 ps for G in the same solvent. The fit parameters in Table 1 show that ground-state recovery occurs on average more than an order of magnitude more slowly in G·C duplexes than the fluorescence lifetime seen for a single GC base pair by Schwalb and Temps.[11]

Although all oligomers in Figure 1 contain Watson−Crick G·C base pairs, the decay times depend on whether the stacked base pairs are alternating as in $d(GC)_9 \cdot d(GC)_9$, or nonalternating as in $d(C_4G_4) \cdot d(C_4G_4)$ and the two hairpins. The strong dependence on base sequence along a strand (alternating vs nonalternating) is readily explained by a model in which initial excitons decay to intrastrand exciplexes.[2,19] This study and our previous study[2] of A·T DNA photophysics highlight the importance of vertical base stacking interactions, but future work is needed to explore the precise effects of base pairing on nonradiative decay pathways.

In summary, ground-state repopulation following UV excitation in various GC systems occurs more slowly, and not more rapidly, than in the constituent mononucleotides. Ultrashort fluorescence lifetimes and slow ground-state recovery are explained by a model in which fluorescent excited states rapidly and irreversibly decay to "dark" excited states. Available evidence suggests that most excited states decay via intrastrand exciplexes and not via interstrand proton transfer. Long-lived excited states have been observed in A tracts,[5] AT-oligonucleotides,[2] C tracts,[20] dinucleosides,[19] and now in GC-oligonucleotides. These systems include single-, double-, and even multistranded forms. The finding that electronic relaxation to the ground electronic state takes place more slowly in base multimers than in monomeric bases is clearly a general result that holds under diverse conditions of base stacking and base pairing. The photochemical consequences of the dark excited states must still be determined, but it is clearly erroneous to ascribe DNA's photostability to subpicosecond ground-state recovery as in single bases. On balance, excited states evolve remarkably differently in base monomers and multimers. The ability to differentiate nucleic acid structures based on excited-state dynamics could open up exciting opportunities for probing time-evolving structures in nucleic acids.

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Supporting Information Available: Experimental methods and all fitting parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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