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Deuterium Isotope Effect on Excited-State Dynamics in an Alternating GC Oligonucleotide

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Understanding how base pairing and stacking mediate the dissipation of electronic energy in DNA is essential for understanding the initial steps in UV photodamage. Excited states of individual DNA bases in solution decay to the ground state either directly by ultrafast internal conversion or, in the case of pyrimidine bases, indirectly via longer-lived triplet and $^1n\pi^*$ states.[1] The base stacking present in single- and double-stranded DNA causes these systems to have dramatically longer excited-state lifetimes than monomeric bases.[2−5] Femtosecond transient absorption experiments have detected long-lived excited states in numerous π-stacked systems from dinucleosides[5] to G-quadruplexes.[1] These long-lived states are formed in high yields only when π stacking is present and are observed in stacks composed of both AT and GC base pairs.[3,4,6] A recent model assigns these states to charge-transfer (CT) excited states (exciplexes) formed between π-stacked bases that arise from initially populated Frenkel exciton states.[3−5] Although base stacking is clearly a requirement for the formation of these long-lived states, the consequences of base pairing for DNA excited-state dynamics is still highly uncertain and provided the motivation for this study.

The possibility that UV mutagenicity is a consequence of proton transfer between paired bases was proposed many years ago.[7] More recently, proton transfer was suggested to be responsible for the photostability of DNA.[8−10] Using IR−UV hole-burning spectroscopy, Abo-Riziq et al.[11] observed a broad UV spectrum for isolated Watson−Crick (WC) GC base pairs in the gas phase, whereas sharp UV spectra were observed for non-WC GC base-pairing combinations. The broad UV spectrum unique to GC base pairs in the WC conformation was suggested to be the result of lifetime shortening due to a proton transfer mechanism. Subsequent ab initio calculations implicated an ultrafast deactivation pathway between the excited $^1\pi\pi^*$ state and the ground state mediated by proton transfer.[9,12] Recently, Schwalb and Temps[13] reported shortened fluorescence lifetimes in isolated WC GC base-pair analogues relative to the monomers in chloroform using fluorescence up-conversion spectroscopy. However, because these model systems lack π-stacking interactions, their relevance to duplex DNA is uncertain.

In this work, we investigated the effect of base pairing on the excited-state dynamics in GC-containing duplexes when base stacking is also present. This study is timely in view of recent reports of inter- and intrastrand CT states in computational studies of excited states in double-stranded DNA.[9,12,14−16] We report the discovery of a pronounced isotope effect on the excited-state lifetimes in an alternating GC oligonucleotide, demonstrating that interstrand hydrogen bonds can significantly affect the excited-state dynamics in double-stranded DNA.

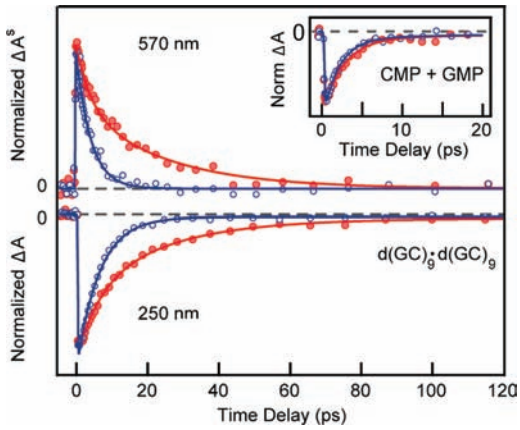

Figure 1. Normalized transient absorption signals showing (top) excited-state absorption and (bottom) ground-state bleach recovery of $d(GC)_9 \cdot d(GC)_9$ in H_2O (blue ○) and D_2O (red ●). The inset shows the 250 nm transient for an equimolar mixture of the monomers CMP and GMP in H_2O (blue ○) and D_2O (red ●). The signals at 570 nm were corrected for solvated electrons using the procedure described previously.[2] The solid curves are nonlinear least-squares fits to the data.

Transient absorption signals were recorded for $d(GC)_9 \cdot d(GC)_9$ at an excitation wavelength of 266 nm and probe wavelengths of 250 and 570 nm in H_2O and D_2O, as shown in Figure 1. These data show that there is significantly faster ground-state recovery for $d(GC)_9 \cdot d(GC)_9$ in H_2O than in D_2O. In contrast, only a minor isotope effect (Figure 1 inset) was observed for the more rapidly decaying signals from an equimolar mixture of the 5′-mononucleotides, CMP and GMP. The circular dichroism spectra for $d(GC)_9 \cdot d(GC)_9$ in H_2O and D_2O are identical (Figure S1 in the Supporting Information), indicating that replacement of exchangeable hydrogens by deuterium atoms does not measurably perturb the duplex structure. The observed isotope effect is thus not the result of a change in secondary structure.

In H_2O, the transient signals decay monoexponentially with time constants of 4.1 ± 0.3 and 6.3 ± 0.4 ps for probe wavelengths of 570 and 250 nm, respectively (Table 1). Measurements at 250 nm monitor ground-state repopulation after excitation, whereas probing at 570 nm reports on excited-state populations.[17] The longer time constant observed at 250 nm than at 570 nm likely results from vibrational cooling following fast relaxation from an excited state to the ground state.[18] These lifetimes, which are approximately an order of magnitude longer than the fluorescence lifetimes of CMP and GMP,[1] are assigned

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Table 1. Time Constants (τ) and Percent Amplitudes (in Brackets) from Global Fits to Transient Signals of $d(GC)_9 \cdot d(GC)_9$ in H_2O and D_2O[a,b]

solvent	λ_{probe} (nm)	τ_1 (ps)	τ_2 (ps)
H_2O	570	4.1 ± 0.3 [100]	–
	250	6.3 ± 0.4 [98]	–
D_2O	570	4.1 ± 0.3 [41]	22 ± 4 [58]
	250	6.3 ± 0.4 [41]	22 ± 4 [56]

[a] Stated uncertainties are twice the standard error; parameters with identical values and uncertainties were globally linked during fitting. [b] Amplitudes do not sum to 100% because of a small amount of residual photobleaching.

Table 2. Time Constants (τ) and Percent Amplitudes (in Brackets) from Global Fits to Transient Absorption Signals (266 nm Pump/250 nm Probe) for Various DNAs[a,b]

system	solvent	τ_1 (ps)	τ_2 (ps)
CMP + GMP	H_2O	2.0 ± 0.3 [92]	31 ± 26 [8]
	D_2O	2.6 ± 0.4 [91]	31 ± 26 [9]
$d(C_4G_4) \cdot d(C_4G_4)$	H_2O	3.1 ± 0.7 [61]	22 ± 5 [32]
	D_2O	3.1 ± 0.7 [60]	22 ± 5 [33]
$d[(GX)_9GC]$	H_2O	5.1 ± 1.0 [72]	25 ± 5 [25]
	D_2O	6.6 ± 2.0 [55]	25 ± 5 [43]

[a] Stated uncertainties are twice the standard error; parameters with identical values and uncertainties were globally linked during fitting. [b] Amplitudes do not sum to 100% because of a small amount of residual photobleaching.

to long-lived exciplex states.[4] A small constant offset was seen at longer delay times at 250 nm and is assigned to a minor amount of photobleaching.

In D_2O, the signals initially decayed with the same time constants found in H_2O (τ_1 in Table 1), but an additional, approximately equal-amplitude component with a lifetime of 22 ± 4 ps was also detected (Table 1). In UV−IR experiments on the closely related alternating copolymer poly[d(GC)]·poly[d(GC)] in D_2O, Doorley et al.[19] observed biexponentially decaying signals with lifetimes of 7 ± 1 and 30 ± 4 ps and approximately equal amplitudes. These values agree well with our observations for $d(GC)_9 \cdot d(GC)_9$ in D_2O probed at 250 nm. The similarity between the UV−UV and UV−IR bleach recovery signals for DNA systems was noted previously.[20]

Doorley et al.[19] argued that the slow component is due to a $^1n\pi^*$ state of 2′-deoxycytidine. They further suggested that the absence of a slow decay in our earlier UV−UV measurement[4] for $d(GC)_9 \cdot d(GC)_9$ in H_2O could have been due to difficulties detecting a $^1n\pi^*$ state by transient absorption spectroscopy. However, the signals in Figure 1 clearly show that the 22 ps component is easily observed in D_2O but absent in H_2O. Furthermore, the presence of the 22 ps component at both the 250 and 570 nm probe wavelengths for $d(GC)_9 \cdot d(GC)_9$ in D_2O rules out the assignment of the long-lived state to a $^1n\pi^*$ state because nucleobase $^1n\pi^*$ states do not absorb at visible wavelengths.[17]

A deuterium kinetic isotope effect is observed in pump−probe experiments on single nucleotides as a result of different rates of vibrational cooling in H_2O and D_2O following ultrafast ground-state repopulation.[18] This is the reason for the modest kinetic isotope effect (τ_D/τ_H) of 1.3 seen in the equimolar CMP/GMP mixture (Table 2). However, vibrational cooling of a hot ground state is not detectable at a probe wavelength of 570 nm, so the isotope effect seen in $d(GC)_9 \cdot d(GC)_9$ must have a different origin.

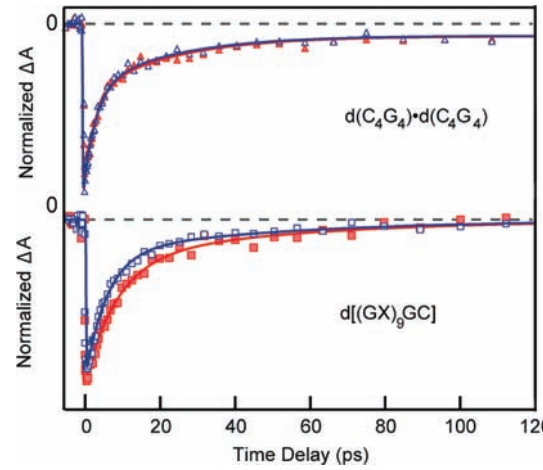

Figure 2. Normalized transient absorption signals (266 nm pump/250 nm probe) of (top) $d(C_4G_4) \cdot d(C_4G_4)$ and (bottom) $d[(GX)_9GC]$ in H_2O (open markers) and D_2O (solid markers).

In order to test the hypothesis that the isotope effect is due to interstrand proton transfer, experiments were carried out on $d[(GX)_9GC]$, where X is 3-methylcytidine. Methylation of C at N3 prevents WC base pairing with G.[21] The bleach-recovery signals of single-stranded $d[(GX)_9GC]$ in H_2O and D_2O (Figure 2 bottom) show that suppressing the base pairing between strands with alternating GC bases eliminates the pronounced dynamical differences seen for $d(GC)_9 \cdot d(GC)_9$ in H_2O and D_2O.

The $d[(GX)_9GC]$ signals at 570 nm (Figure S2) and 250 nm were fit to a function containing two exponentials and a time-independent offset. As shown in Table 2, a 5−7 ps component was found along with an additional 25 ± 5 ps component that agrees well with the 22 ± 4 ps lifetime found for $d(GC)_9 \cdot d(GC)_9$ in D_2O. This lifetime, which is similar to the value of 12 ± 8 ps reported for the RNA dinucleoside monophosphate CpG,[5] is assigned to an intrastrand exciplex state. The kinetic isotope effect of 1.3 observed for the fast decay component of $d[(GX)_9GC]$ is consistent with vibrational cooling, which could be a consequence of rapid deactivation of monomer-like excited states formed in places where bases are less well stacked in this single-stranded form.

The lack of an isotope effect in $d[(GX)_9GC]$, where base pairing is absent, strongly suggests that an interstrand process contributes to the excited-state dynamics of $d(GC)_9 \cdot d(GC)_9$. Significantly, no isotope effect was observed for the nonalternating GC duplex $d(C_4G_4) \cdot d(C_4G_4)$ (Figure 2 top and Table 2). These findings parallel previous results on AT-containing DNAs, where a solvent kinetic isotope effect was observed in aqueous solutions of $d(AT)_9 \cdot d(AT)_9$ but not for the nonalternating duplex $d(A)_{18} \cdot d(T)_{18}$.[3]

The fact that an isotope effect is seen in $d(GC)_9 \cdot d(GC)_9$ but not in $d(C_4G_4) \cdot d(C_4G_4)$, which has the identical base-pairing motif, indicates that the quenching mechanism is not restricted to interactions within a single base pair but instead must involve a pathway that is additionally mediated by base stacking. Crespo-Hernández et al.[3] proposed that the isotope effect observed in alternating $d(AT)_9 \cdot d(AT)_9$ results from interstrand proton transfer initiated by the formation of an intrastrand exciplex state. The present results lend support to the concept that exciplex states with significant CT character enable proton transfer across base pairs. The exciplex state formed by the alternating G and C bases in $d(GC)_9 \cdot d(GC)_9$ is expected to have stronger CT character than

that in $d(C_4G_4)\cdot d(C_4G_4)$.[5] In $d(C_4G_4)\cdot d(C_4G_4)$, which contains just a single 5′-CpG-3′ step, the signal is dominated by excimer states formed in CC and/or GG stacks. The latter states may lack sufficient charge separation to drive interstrand proton transfer. Alternatively, as suggested by a reviewer, charge delocalization over like bases in each strand of $d(C_4G_4)\cdot d(C_4G_4)$ could prevent the degree of charge localization needed to induce proton transfer.

The formation of an intrastrand exciplex state with strong CT character between stacked cytosine and guanine is expected to give guanine cationic character and cytosine anionic character, while each remains base-paired to its neutral complementary base. Importantly, the barriers for proton transfer have been predicted to be lowered in both the one-electron-oxidized and one-electron-reduced GC base pairs.[22−24] Calculations by Li and Sevilla[23] predicted that proton transfer for the GC radical anion base pair is energetically favorable, with a free-energy change of −3 kcal/mol and a small activation barrier of 1 kcal/mol. However, proton transfer in the isolated GC radical cation base pair was predicted to be slightly energetically unfavorable.[23] Earlier, Bertran et al.[22] predicted that the single proton transfer reaction is endergonic for the GC radical cation base pair, with a calculated barrier of 4.3 kcal mol^{-1}.

Kumar and Sevilla[24] found that including water molecules to mimic the hydration environment in duplex DNA lowers the predicted free-energy change to −0.65 kcal mol^{-1} and gives an activation energy of 1.42 kcal mol^{-1} for the GC radical cation base pair, making proton transfer favorable. Recent experimental work by Sevilla and co-workers[25] on double-stranded DNA has provided evidence that one-electron-oxidized GC base pairs exist in the deprotonated neutral radical form following interbase proton transfer at 77 K. These studies supporting the feasibility of proton transfer in radical ion base pairs make it plausible that interstrand proton transfer could occur either in concert with or sequential to intrastrand electron transfer initiated by UV absorption.

In summary, a pronounced isotope effect has been observed in $d(GC)_9\cdot d(GC)_9$ but not in nonalternating or single-stranded GC-containing DNAs. The dynamics suggests that proton-coupled electron transfer is an important decay pathway in duplex DNAs having an appropriate base sequence. Proton transfer is thus capable of mediating excited-state decay (i.e., electron−hole recombination) in DNA as well as the rate of hole transport through DNA.[26,27] Further work is needed to obtain direct evidence for proton transfer, determine any long-time photoproducts that may be produced, and ascertain the time scales for electron and proton motion.

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

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