Femtosecond UV and Infrared Time-Resolved Spectroscopy of DNA: From Well-ordered Sequences to Genomic DNA

DISSERTATION

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Abstract

DNA contains the genetic code for all life. The nucleic acid bases that comprise DNA readily absorb ultraviolet light, making them susceptible to photodamage that can lead to mutations and skin cancer. Knowledge of the pathways by which excess electronic energy is dissipated in DNA is important for understanding how photodamage to DNA occurs. The aim of this work is to understand the effect of sequence and secondary structure on the excited-state dynamics in a diverse set of DNA systems.

The excited-state dynamics of various GC-containing oligonucleotides were studied by femtosecond transient absorption spectroscopy. Long-lived states with lifetimes at least an order of magnitude slower than the constituent monomers were observed in all the systems studied. Significantly altering the structural conformation of d(GC)$_9$·d(GC)$_9$ through the use of high ionic conditions and low pH revealed that long-lived excited state lifetimes were nearly independent of base stacking geometries and base pairing motifs. The electronic coupling for charge recombination in the long-lived exciplex states of d(GC)$_9$·d(GC)$_9$ is proposed to be large and not significantly altered by helix conformation. The driving force, rather than electronic coupling, for charge recombination is proposed to influence strongly the decay of the long-lived exciplex states in this duplex.

Transient absorption experiments were carried out on a genomic DNA sequence to study the effect of sequence disorder on excite-state dynamics. Long-lived excited
states were observed in the individual single strands as well as in the duplex of the ras6l oligodeoxynucleotide. The lifetimes of the individual strands were sensitive to composition, with a slower decay observed for the purine-rich strand compared to the pyrimidine-rich strand. In addition, a large ultrafast decay component was observed in the duplex suggesting monomer-like decay pathways are accessible in well-stacked DNA systems. This study shows that long-lived excited states are not an artifact of well-ordered model systems, but are also present in genomic DNA.

Isotopic studies revealed a pronounced deuterium isotope effect on the excited-state dynamics of alternating GC-containing oligonucleotides that is not present in non-alternating GC-duplexes. This result parallels those of similar AT-DNAs and is proposed to involve interstrand proton transfer initiated by the formation of an exciplex state with significant charge transfer character. To investigate further the possibility of interstrand proton transfer in DNA duplexes, a series of A- and T-containing oligonucleotides were studied by UV-pump / mid-IR probe spectroscopy. Unique, strong positive bands are observed in alternating AT-DNA, but not in the single- and double-strand non-alternating AT-oligomers. These bands are proposed to be marker bands for proton transfer. Possible absorption bands for excimer states were also observed in (dA)$_{18}$ in the spectral region of 1500 - 1800 cm$^{-1}$, supporting previous measurements on polyA.
Dedication

This work is dedicated to my husband for all his encouragement and support.
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I would like to thank my advisor, Bern Kohler, for his encouragement, insightful conversations and always-optimistic outlook.

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Chapter 1: Introduction

The most common form of cancer is skin cancer, with more than 2 million cases diagnosed each year in the United States. This form of cancer is caused by prolonged exposure to ultraviolet (UV) light. Given the alarming rates of skin cancer, there is much interest in understanding the processes by which exposure to UV light can lead to mutagenic photoproducts in DNA. The chromophores that make up DNA, cytosine (C), guanine (G), adenine (A) and thymine (T) (Figure 1.1), absorb strongly in the UV region of the spectrum. Understanding the relaxation pathways for excess electronic energy deposited in the DNA by UV photons is essential for understanding UV photodamage.

Many studies have focused on understanding the photophysics of the fundamental building blocks that make up DNA. Initial time-resolved studies revealed that following UV excitation to the $^1\pi\pi^*$ state, DNA bases undergo ultrafast nonradiative decay back to the ground state in less than a picosecond. Theoretical studies identified conical intersections (CIs) that provide a barrierless pathway for internal conversion from the excited state to the ground state in all the natural bases. The ability of these bases to rapidly dissipate excess electronic energy reduces the possibility to undergo photochemistry, making them more photostable. It has been proposed that the photostability of the DNA bases played a role in their preferential selection as the
building blocks of our genetic material.\textsuperscript{2,13,14} Recent studies have revealed that the excited-state landscape in DNA is more complicated. When multiple DNA base are arranged together to form higher-ordered structures with base stacking and pairing interactions, long-lived excited states with lifetimes of tens of picoseconds and longer have been observed.\textsuperscript{15-26,27} There is much interest in how the structural components of DNA influence the excited-state dynamics.

Femtosecond transient absorption studies have reported long-lived excited states in high yields in numerous $\pi$-stacked systems from minimally stacked dinucleosides\textsuperscript{28} to genomic DNA.\textsuperscript{29} Studies on single-stranded homo-adenine systems report excited state decay on two distinct time scales: an ultrafast decay similar to that seen in adenine and a slower hundred picosecond decay not observed in the base monomers.\textsuperscript{25,30} The fast decay was assigned to monomer-like pathways in unstacked or poorly stacked regions, while the slow decay was assigned to long-lived states formed in regions of base stacking (Figure 1.2). The nature of these long-lived states has been a point of controversy: some studies assigned the states to intrastrand charge-transfer states or exciplexes formed between $\pi$-stacked bases (following the early work of Eisinger et al.\textsuperscript{31} on DNA glasses),\textsuperscript{28} and others assigned them to excitonic states delocalized over at least two bases.\textsuperscript{19,32} A study of a series of dinucleosides reported that the decay lifetime correlates with the energy of the proposed charge-transfer state, supporting the assignment of these states to exciplexes.\textsuperscript{28} Theoretical studies have also provided support for the assignment of these states to charge-transfer states.\textsuperscript{33-36} A recent model proposes that excitonic states delocalized over at least two bases are formed initially following excitation and
dynamically evolve to exciplex states localized over a pair of \( \pi \)-stacked bases in less than a picosecond.\(^3\)

Although the importance of base stacking to long-lived excited states has been established in A-containing DNA, less is know about how base pairing affects the excited-state dynamics. A study on single- and double-stranded AT-containing DNA showed that no significant change occurred in the presence of base pairing interactions.\(^{25}\) These results indicate that base stacking, and not base pairing, interactions strongly influence the relaxation of electronic energy in AT-containing DNA. However, a pronounced kinetic isotope effect has been reported for alternating AT and GC duplexes, suggesting that interstrand processes also play a role in the excited-state dynamics.\(^{22,25}\) Crespo-Hernández et al. proposed that exciplex states with significant charge transfer could undergo intrastrand proton transfer.\(^{25}\)

Proton transfer has been proposed to be both a source of mutations within DNA\(^{37}\) and responsible for the photostability of DNA.\(^{38-40}\) Gas-phase experiments by Abo-Riziq et al. suggested that ultrafast proton transfer may quench excited states in Watson-Crick (WC) GC base pairs, but not in non-WC base pairs.\(^{41}\) Schwalb and Temps reported quenching of fluorescence lifetimes in WC GC base pair analogs relative to the isolated monomers in non-polar chloroform using fluorescence up-conversion spectroscopy.\(^{42,43}\) Similar results were also reported for poly(dGdC)-poly(dGdC) in solution.\(^{44}\) Theoretical studies reported an ultrafast deactivation pathway mediated by proton transfer in both GC\(^{39,45}\) and AT base pairs.\(^{46}\) Despite these studies, there is no direct evidence for proton transfer in UV-excited single- or double-stranded DNA.
The main objectives of the studies reported in this dissertation are to understand the effect of sequence, stacking geometries, and base pairing interactions on the excited-state dynamics of DNA oligonucleotides. Base sequence has been shown to play an important role in the excited-state dynamics in single- and double-stranded DNA.\textsuperscript{16,25,28} Questions have been raised as to whether long-lived excited states similar to those observed in model A- and AT-containing oligonucleotides are also present in GC-containing oligonucleotides and systems with significant sequence disorder.\textsuperscript{16,19,42} One of the aims of this work is to determine whether long-lived excited states are present in these systems and how sequence affects the excited-state dynamics. With the exception of a few studies,\textsuperscript{15,17,26} most time-resolved spectroscopy studies have focused on how base pairing and stacking influences the excited-state dynamics without addressing the polymorphism of DNA. DNA is a flexible system able to adopt different conformations and structures based on the solvent environment.\textsuperscript{47} Base conformation has been shown to be important for charge transfer in DNA.\textsuperscript{48,49} Another aim of this dissertation work is to investigate the effect of different base stacking geometries and base pairing motifs on excited-state dynamics in DNA duplexes. Finally, proton transfer in DNA has been of interest for many years.\textsuperscript{38-40} Direct evidence of proton transfer within UV-excited double-stranded DNA is lacking. A final goal of this dissertation work is to provide conclusive evidence for interstrand proton transfer in DNA. Understanding the role of sequence, base stacking and base pairing in the dissipation of excess electronic energy in DNA will provide important insight into the connection between ultraviolet exposure and DNA damage.
This dissertation is organized as follows: Chapter 2 describes the experimental setups and methods. Particular attention is given to the discussion of sample handling for the UV-pump/mid IR-probe experiments. In Chapter 3, transient absorption experiments on various GC-containing oligonucleotides are described. Long-lived excited states are reported for all the oligonucleotides studied. The effect of base stacking geometry and base pairing motif on the long-lived excited state lifetimes are discussed. A pronounced isotope effect is observed and proposed to involve proton transfer initiated by the formation of exciplex states with significant charge transfer. Chapter 4 covers experiments on genomic sequences with significant sequence disorder. The observation of long-lived states in single- and double-stranded genomic sequences shows that long-lived states are not an artifact of well-ordered, model DNA systems. Possible quenching of long-lived excited states and a fast deactivation pathway in the well-stacked duplex are also discussed. Mid-IR transient absorption studies on a series of A- and T- containing oligonucleotides are presented in Chapter 5. Spectral features of the AT-containing oligonucleotides are compared and possible marker bands for excimer states and proton transfer are assigned. Chapter 6 discusses preliminary results of selective excitation experiments on d(G₃A₄G₃).
References


(49) O’Neill, M. A.; Becker, H.-C.; Wan, C.; Barton, J. K.; Zewail, A. H.

Figure 1.1. Structures of the DNA base monomers.
Figure 1.2. Proposed energy diagrams for a) base monomers and b) stacked DNA bases.
Chapter 2: Experimental Methods

2.1. Transient Absorption Spectroscopy

The foundation of the transient absorption measurements discussed in this chapter is an amplified femtosecond Ti:Sapphire laser system. This system and the accompanying spectrometers are part of the Center for Chemical and Biophysical Dynamics at The Ohio State University. The amplified Ti:Sapphire laser system consists of a mode-locked femtosecond Ti:Sapphire oscillator (Mira, Coherent, Inc., Santa Clara, CA, pre-2010; Mantis, Coherent, Inc., Santa Clara, CA, post-2010) and two chirped-pulse amplifiers (Legend-HE, Coherent, Inc., Santa Clara, CA). The oscillator, pumped by a diode-pumped solid state Nd:VO$_4$ laser (Verdi-V5, Coherent, Inc., Santa Clara, CA) at 532 nm, generated pulses that were typically around 500 - 800 mW, centered at 795 nm, with a FWHM of 45 nm, and a repetition rate of 76 MHz. The output pulses are split to seed two chirped-pulse amplifiers. The amplifiers consist of three parts: a stretcher, a regenerative amplifier pumped by a Q-switched Nd:YLF diode laser (Evolution-30, Coherent, Inc., Santa Clara, CA), and a pulse compressor. The output pulses were centered at 800 nm, with typical pulse energies of 2.4 mJ, a FWHM of 60 fs, and a repetition rate of 1 kHz. One amplifier generated the pump and probe pulses for the UV-pump / UV-probe and UV-pump / visible-probe experiments described in Section 2.2.
The other amplifier produced the pulses used in the UV-pump / mid-IR probe transient absorption experiments discussed in Section 2.3.

2.2. UV-pump /UV-probe and UV-pump /Visible-probe Measurements

The experimental setups for the UV-pump / UV-probe and UV-pump / visible-probe spectrometers are shown in Figure 2.1. Output pulses from the amplified Ti:Sapphire system was split by a 70/30 beam splitter, with 70% of the power being reflected to two optical parametric amplifiers (OPA) (OperA, Coherent, Inc., Santa Clara, CA) and 30% passing through for the third harmonic (THG) and white light continuum (WLC) generation paths.

Pump pulses at 266 nm were obtained from THG. Briefly, the 800 nm fundamental from the amplified Ti:Sapphire laser system was reduced in beam size and collimated by a telescope. This beam passed through a beam splitter, with 25% of the power reflected to a delay stage to enable temporal overlap in the THG crystal and a half waveplate to rotate the polarization by 90°, and the remaining 75% of the power was frequency doubled in a 2-mm type-I BBO (30°) crystal. The 400 nm and 800 nm beams were spatially and temporally overlapped in a 1-mm type-II BBO crystal (45°), leading to sum frequency mixing and the generation of 266 nm light. The resulting beam, containing 266 nm and residual 400 and 800 nm light, was passed through a prism compressor to: 1) spatially filter out the 266 nm light and 2) to shorten the pulse duration.

Pump pulses at 290 nm and probe pulses at 250 nm were generated using OPAs outfitted with an UV/Vis and a SFG harmonics box, respectively. The 800 nm
fundamental from the amplified femtosecond Ti:Sapphire system provided the input beam, which was split evenly between two OPAs. In both OPAs, the input beam was split into 3 paths: a WLC path, a pre-amplifier path, and a power amplifier path. In the WLC path, the 800 nm beam is focused into a stationary sapphire plate to generate WLC. The resulting WLC was collimated and passed into a type-II BBO crystal. The preamplifier beam was overlapped with the WLC both spatially and temporally in the BBO crystal, producing the signal and idler beams. Depending on the crystal angle, the resulting signal beam ranged from 1150 - 1600 nm and the idler beam ranged from 1600 - 2630 nm. The signal and idler beams were passed back through the BBO crystal, and overlapped with the power amplifier for amplification. The amplified signal and idler beams passed into either an UV/Vis or SFG harmonics box to undergo further non-linear processes depending on the desired output wavelength. With the UV/Vis harmonics box, second harmonics and fourth harmonics of the signal and idler beams generated light from 580 - 1150 nm and 285 - 480 nm. Pump pulses at 290 nm were obtained from the fourth harmonic of the signal beam. The SFG harmonics box used sum frequency generation and frequency doubling to produce light from 472 - 533 nm and 236 - 307 nm. The second harmonic of the sum frequency between the signal and 800 nm produced the probe pulses at 250 nm.

Visible probe pulses were obtained from white light continuum generation. A fraction of 800 nm fundamental was focused into a stationary 1-cm water-filled quartz cuvette (Starna Cells, Inc., Atascadero, CA) generating a supercontinuum. Specific probe wavelengths were selected after the sample using either a 10-nm interference filter
(Andover Corporation, Salem, NH) or a double-grating monochromator (DH-10, HORIBA Jobin Yvon Inc., Edison, NJ).

The pump beam was passed through an optical chopper followed by a half waveplate and polarizer. The optical chopper was set to block 2 out of every 3 pulses. This reduced exposure of the sample to the pump and provided a modulation in the pump pulses for the lock-in amplifier. The half waveplate / polarizer combination was used to adjust the polarization of the pump beam to be at magic angle (54.7°) with respect to the probe beam to eliminate signals due to reorientation dynamics and adjust the power of the pump beam to be around 1 mW (chopped) at the sample. The pump beam was focused at the sample to a spot size of 1000 - 1500 µm.

The probe pulses were delayed in time with respect to the pump pulses using a computer-controlled delay stage with a retroreflector. Probe beams were focused into the sample to a spot size of 300 - 500 µm. After the sample, UV probe signals were spectrally isolated from UV pump scatter using a double-grating monochromator before detection by a photomultiplier tube. Visible signals were detected with either a photomultiplier tube or a joulemeter (J3S-10, Molelectron). Signals from the detectors were sent to a lock-in amplifier (SR830, Stanford Research Systems, Inc., Sunnyvale, CA) synchronized to the chopper frequency. The difference in probe intensity between the pump on and the pump blocked was measured by the lock-in amplifier and sent to a data collection program written in Labview (National Instruments Corporation, Austin, TX). The data collection program recorded the averaged signal sent from the lock-in at
each time delay along a scan, generating a transient absorption scan. Typically, between 5 and 10 scans were collected and averaged to produce the reported data.

2.2.1. Sample Handling.

Materials. Ammonium salt forms of the DNA oligonucleotides d(C₄G₄), d(C₅A₄G₅), d(C₅T₄G), and d(GC)₉ were obtained from Midland Certified Reagent Company (Midland, TX) as lyophilized powders. AMP (adenosine 5'-monophosphate disodium salt), TMP (thymidine 5'-monophosphate disodium salt hydrate), GMP (guanine 5'-monophosphate disodium salt), CMP (cytidine 5'-monophosphate disodium salt) and D₂O (deuterium oxide, 99.9 atom % D) were purchased from Sigma-Aldrich Co., USA) and used as received.

Sample Preparation. Samples for the H₂O measurements were prepared in 50 mM aqueous phosphate buffer (pH 6.8) containing 0.25 M NaCl. Phosphate buffer was prepared from 25 mM Na₂HPO₄ (sodium phosphate dibasic anhydrous) and 25 mM KH₂PO₄ (potassium phosphate monobasic) dissolved in NANOpure water (NANOpure Ultrapure Water System, Barnstead International, Dubuque, IA). Measurements in D₂O were carried out on samples dissolved in unbuffered D₂O containing 0.25 M NaCl. A pH value of 6.7 was measured for the unbuffered D₂O salt solutions using an Accumet Model 15 pH meter (Fisher Scientific, Pittsburg, PA) with a double junction, Ag/AgCl glass pH electrode.¹ This pH is equivalent to a pD value of 7.1.¹ For high-salt experiments discussed in Section 3.2.2, d(GC)₉ was dissolved in 25 mM aqueous phosphate buffer containing 5.0 M NaCl. For low pH experiments discussed in Chapter 3, the Hoogsteen-
base-paired d(GC)$_9$·d(GC)$_9$ duplex was formed by adding dilute HCl dropwise to deionized water containing 0.25 M NaCl until pH 3.6 was reached.

Non-complementary duplex solutions were made by mixing equimolar solutions of each strand. All duplexes were thermally annealed by heating to 70°C for 10 minutes followed by slow cooling back to room temperature. For pump-probe experiments, concentrations were adjusted to obtain an absorbance of 1 at the pump wavelength of 266 nm. Samples were held between two 1-mm thick CaF$_2$ windows separated by a 1.2-mm thick Gylon spacer (Fluidol, Columbus, OH) in a home-built spinning cell and spun at several hundred RPM to avoid re-excitation of the sample. Samples were monitored for photodegradation by UV/Vis absorption spectroscopy during the course of the experiment.

2.3. UV-pump/Mid-IR probe Measurements

The experimental setup for the UV-pump / mid-IR probe spectrometer is shown in Figure 2.2. Output pulses from the second Ti:Sapphire amplifier was split evenly between two OPAs. UV pump pulses at 270 ± 2 and 290 ± 2 nm were generated using an OPA with a SFG harmonics box as described in Section 2.2. The UV-pump beam passed through an optical chopper and shutter before being sent to a computer-controlled delay stage that delayed the pump pulses in time. The pump beam passed through a waveplate set at magic angle with respect to the probe beam and was focused to a spot size of 500 - 800 µm at the sample.
An OPA with a DFG harmonics box produced the mid-IR pulses by difference frequency generation between the signal and idler beams. The OPA-DFG had a spectral range of 4166 - 1000 cm\(^{-1}\). The UV-pump / mid IR-probe experiments discussed in Chapter 5 and 6 used mid-IR probe pulses in the range of 1500 - 1800 cm\(^{-1}\). These pulses generally had a power of 2 - 3 mW and a FWHM of 100 cm\(^{-1}\). A 2500 ± 100 nm long pass filter (LP-2500 nm, Spectogron) removed the residual signal and idler wavelengths from the beam. The DFG harmonics box and TRIR optics were enclosed in a plexiglass box filled with dry air to limit absorption of the IR pulses by water vapor. The mid-IR probe beam was split into two paths by a 50/50 beam splitter: a probe path and a reference path. The probe path was focused into the sample, re-collimated after the sample and then focused into the entrance of the spectrometer (Jobin-Yvon Triax 320). The reference beam was lowered in height by 5 mm with respect to the probe beam before being focused into the sample directly below the probe beam. It was re-collimated after the sample and focused into the entrance slit of the spectrometer 5 mm below the probe beam. A variable circular neutral density (ND) filter (ND 0-2, zinc selenide substrate) placed directly after the OPA output was used to adjust the intensity of the mid-IR pulses. ND filters placed in either the probe or reference beams after the sample were used to achieve similar probe/reference beam intensities at the detector.

For rough spatial overlap of the pump and probe beams at the sample, an iris was used. The iris was centered on the IR probe beam by monitoring the array signal and maximizing the signal by adjusting the vertical and horizontal position of sample stage. Once the iris was centered on the IR probe beam, the UV pump beam was roughly
centered on the iris by eye. To find temporal overlap, a Ge window was placed at the sample position, and the delay stage was scanned to find a rough time zero using the strong signal from Ge. Adjusting the pump beam position to maximize the signal from the Ge window also improved the spatial overlap between pump and probe. After the sample was placed in the holder, the position of the pump beam was adjusted to optimize the amplitude of the features of the transient spectra at a delay time of 1ps. A time window from -3 to 3 ps was scanned to determine the position of time zero for the experiments.

The spectrometer was equipped with 3 gratings with different wavelength ranges and resolutions. The grating (Grating 3) used in the UV-pump / mid IR-probe experiments discussed in Chapter 5 and 6 had 50 grooves per mm and a blaze wavelength of 6.0 µm. It had a spectral range of 1111 to 2500 cm\(^{-1}\), a spectral window of 100 cm\(^{-1}\) and a minimum spectral resolution of 3 cm\(^{-1}\). The spectrometer was coupled with a liquid-nitrogen cooled, dual 32-element array, mercury-cadmium-telluride (MCT) detector (InfraRed Associates). The data collection program used was a custom-built software program written in Labview (National Instruments Corporation, Austin, TX). Calibration of the spectra was done using either water vapor or zero order calibrations.

2.3.1 Sample Handling

Materials. Sodium salt forms of the DNA oligonucleotides d(G\(_3\)A\(_4\)G\(_4\)), (dA)\(_{18}\), (dT)\(_{18}\), and (dAdT)\(_9\) were obtained from Midland Certified Reagent Company (Midland, TX) as lyophilized powders. AMP (adenosine 5'-monophosphate disodium salt), TMP (thymidine 5'-monophosphate disodium salt hydrate) and D\(_2\)O (deuterium oxide, 99.9
atom % D) were purchased from Sigma-Aldrich and used as received. The NaCl used in these experiments was dried in an oven overnight and stored in a desiccator.

Sample Preparation. For experiments discussed in Chapter 5, the samples were prepared in 100 mM phosphate D2O buffer. (dA)₁₈ and (dT)₁₈ were prepared in phosphate D₂O buffer without NaCl. (dAdT)₉ was prepared in phosphate D₂O buffer with 0.25 M NaCl. (dA)₁₈·(dT)₁₈ was prepared in phosphate D₂O buffer and 1 M NaCl in order to increase the melting temperature of the duplex. For experiments discussed in Chapter 6, samples of d(G₃A₄G₃) were prepared in 100 mM phosphate D₂O buffer with no salt added. Samples for the mid-IR transient absorption experiments were prepared to have an absorbance of 0.5 - 1 OD for experiments with a pump wavelength of 270 nm and 0.2 - 0.5 OD for a pump wavelength of 290 nm. Samples had approximately twice the oligonucleotide concentration in the 290 nm pump experiments compared to 270 nm experiments.

Sample Artifacts. Two problems were discovered in initial TRIR experiments on DNA oligonucleotides: counterion contamination and low pD. A strong vibrational band at 1674 cm⁻¹ was observed in all the oligonucleotides samples obtained from Midland. An example of this band in the FTIR spectrum of (dAdT)₉ is shown in Figure 2.3. This band is not observed in published DNA spectra² and was not present in similar samples obtained from Sigma-Aldrich, also shown in Figure 2.3. The oligonucleotide samples obtained from Midland Certified Reagent Company contained ammonium counterions while the samples obtained from Sigma-Aldrich contained sodium counterions. Although the ammonium ion in aqueous solution does not have vibrational bands in this
replacement of the ammonium ions with sodium ions in subsequent Midland samples eliminated this extra band, shown in Figure 2.3. The absence of this band in samples with sodium counterions supports that the vibrational band is linked to the ammonium counterions. This band is likely not the result of a change in solution pH caused by the presence of the weakly acidic ammonium ions. The band position is independent of the sample; the vibrational band at 1674 cm\(^{-1}\) is present in (dA\(_{18}\), (dT\(_{18}\), and (dAdT)\(_{9}\) from Midland. If the band were a result of protonation of the DNA sample, the frequency should change with different DNA samples. In addition, the amplitude of this band is significantly stronger than the vibrational bands assigned to the DNA. As discussed in the next paragraph, the amplitude of vibrational bands arising from base protonation would be expected to be comparable or weaker than the neutral sample. The large amplitude of the vibration band compared to the underlying DNA bands suggesting that the source has a significant concentration in the sample, and the position of the band is independent of the DNA composition suggesting that it does not arise from an interaction with the DNA bases. The vibration responsible for the band at 1674 cm\(^{-1}\) was not determined, but it is proposed to arise from interactions between the phosphate groups and the ammonium counterions rather than changes in solution pH. All subsequent time-resolved infrared experiments (TRIR) experiments used sodium salt forms of the oligonucleotides.

Initial TRIR experiments were carried out using the sodium salt form of Midland oligonucleotides prepared in pure D\(_2\)O with NaCl. Experiments on (dA)\(_{18}\) without buffer contained a vibrational band at 1664 cm\(^{-1}\) that is not present in the published spectrum of
Protonation at the N1 position of adenine causes a shift in the C=C stretching vibration from 1626 to 1668 cm\(^{-1}\) in D\(_2\)O solutions.\(^4\) The similar frequencies for the extra band at 1664 cm\(^{-1}\) in (dA)\(_{18}\) and the C=C stretching vibration for protonated adenines suggests that the (dA)\(_{18}\) solution has a low pD values. To test this hypothesis, experiments were performed in phosphate D\(_2\)O buffer. No vibrational band was observed at 1664 cm\(^{-1}\), confirming that the band is from protonated adenines and that samples in pure D\(_2\)O and NaCl have a low pD solution. Subsequent experiments were performed in phosphate D\(_2\)O buffer. The experiments reported in Chapter 6 used a combination of a BaF\(_2\) window front window and a CaF\(_2\) back window in order to minimize the coherent signals from CaF\(_2\) windows at time zero and because of reports of spectral artifacts from CaF\(_2\) windows. However, it was discovered that phosphate in the buffer forms an insoluble, opaque solid on the surface of the BaF\(_2\) windows. Because of the reaction of phosphate with the BaF\(_2\) windows, CaF\(_2\) windows were used for all the experiments reported in Chapter 5. No spectral artifacts were observed in measurements performed with CaF\(_2\) windows.

2.3.2. IR Spinning Sample Cell

A variable pathlength spinning sample cell, based on the original spinning cell design by Middleton,\(^5\) was constructed for the UV-pump/mid IR-probe transient absorption measurements. Figure 2.5 shows the three parts of the sample cell: a base with a threaded outer wall, an inner ring for compressing the windows, and a cap that threads into the base and secures the assembly. This design allows PTFE (Sigma-Aldrich, St. Louis, MO) and Gylon (Fluidol, Columbus, OH) spacers with thicknesses ranging
from <100 µm to 1.2 mm to be used, making it suitable for UV-pump/mid-IR probe as well as UV/UV and UV/Vis experiments. For experiments with thicker spacers, two injection ports on the side of the cell allow the sample to be injected into the cell using a syringe. For experiments with thinner spacers, the sample was deposited using a dropwise method where the sample is deposited on the surface of a window using a syringe and the second window placed on top of it. The benefit of using a spinning sample cell is the small sample volume needed for experiments compared to flow cells. With a 1.2-mm spacer, approximately 350 µL of sample is required; with a 100-µm spacer, only approximately 40 µL of sample is needed. In contrast, flow cells require sample quantities of at least 1 mL. Higher concentrations of sample are needed for the TRIR experiments to achieve the same optical density as UV/UV and UV/Vis experiments due to the shorter pathlength. A small sample volume makes these experiments more economical.

**Sample Heating.** Samples for UV-pump/mid-IR probe transient absorption experiments were held in a home-built spinning sample cell described in the previous Section. For these experiments, approximately 40 µL of sample solution was held between two 1-inch diameter CaF$_2$ windows separated by a 0.1 mm PTFE spacer (Sigma-Aldrich, St. Louis, MO). The path length of 0.1 mm is 10 times less than that used for transient absorption experiments with UV- and visible-probe pulses and is needed to minimize the strong solvent absorption in the mid-IR. The cell was spun at several hundred RPM to avoid re-excitation of the sample and minimize sample heating. However, significant changes in the duplex spectrum were observed following exposure
to the pump beam. Figure 2.6 shows the transient absorption spectra of \((dA)_{18}'(dT)_{18}\) at a fixed time delay of 1 ps with increasing exposure time to the pump pulses. At early exposure time (12 s), the transient spectrum resembles an inverted ground-state FTIR spectrum of \((dA)_{18}'(dT)_{18}\). As exposure time increases, the bleach band at 1640 cm\(^{-1}\) decreases and merges with the band at 1620 cm\(^{-1}\) to form a broad single band around 1624 cm\(^{-1}\). The red trace curve overlaid on each window in Figure 2.6 is the spectrum of \((dA)_{18}'(dT)_{18}\) after 5 minutes of constant exposure to the pump pulses. The band at 1640 cm\(^{-1}\) is absent from the spectrum at long exposure time and a broad strong bleach band is present at 1624 cm\(^{-1}\) instead. This spectrum more closely resembles the ground-state FTIR spectrum for the individual single-strands \((dA)_{18}\) and \((dT)_{18}\) rather than the duplex, as shown in Figure 2.7. Similar spectral changes are observed for the duplex \((dAdT)_{9}'(dAdT)_{9}\) following prolonged exposure to pump pulses, but not for the single-stranded systems. Photochemistry was ruled out as the reason for the spectral changes because; after 30 minutes without exposure to the pump, the spectrum returns to its original form. Instead, the spectral changes observed in Figure 2.6 are proposed to result from heating of the sample by the pump pulses.

Inadequate sample and energy exchange resulting from the thin path length of the cell are proposed to cause sample heating from the pump pulses. The spectral changes observed in Figure 2.6 for the duplex \((dA)_{18}'(dT)_{18}\) are consistent with those observed during melting of DNA. The steady-state FTIR spectrum duplex \((dA)_{12}'(dT)_{12}\) was recorded as a function temperature from 5 to 70°C by Liquier et al.\(^2\) Distinct spectral changes were observed in the spectral region of 1750 - 1550 cm\(^{-1}\) as a result of DNA
melting, notably the disappearance of the band at 1641 cm\(^{-1}\) and an increase in the adenine band at 1622 cm\(^{-1}\). These changes were assigned to disruption of base pairing interactions at elevated temperatures.\(^2\) The changes observed in Figure 2.6 are similar to those observed by Liquier \textit{et al.}\(^2\) and supports that sample heating is leading to the melting of DNA duplexes in our experiments. The spectrum after 5 minutes of pump exposure (red curve in Figure 2.6) is similar to the (dA)\(_{12}\)⋅(dT)\(_{12}\) at temperatures of 50 °C and greater.\(^2\) The spectrum of the melted DNA duplexes will be discussed further in Chapter 5.

To avoid heating the sample and melting the duplexes, extra measures were taken when working with duplex samples to limit exposure to pump pulses. In addition to spinning the sample cell during data acquisition, the sample holder was manually translated to ensure that a new sample area was probed at each time delay. Transient signals at a limited number (~20) time delays were collected. A specific time delay was selected, the pump shutter was opened and the data acquisition was started. Each scan took approximately 12 s during which time the sample cell was spinning at several hundred rpm. After the scan was complete, the pump shutter was closed, the delay stage was moved to the next time delay, and the sample holder was manually translated to a new position relative to the pump beam. The pump shutter was then opened and data collected at this new time delay. This process was repeated for the desired number of time delays. A new sample was used for each complete data run (ie. after 20 delay times). Using this procedure, the sample was exposed to the pump beam for approximately 6 s (the scan is for ~12 s, but the pump is blocked for half of the scan)
before being moved to a new location in the sample. In contrast, for an average scan from -300 ps to 3 ns, there are 65 delay points and, assuming that the sample is exposed to the pump pulses for only 6 s per scan, the same sample area would be exposed to the pump beam for a total of 6.5 minutes. Although this procedure minimizes sample exposure to the pump pulses, sample heating is still taking place during the time it takes to acquire the data. Comparison between our \((dAdT)_9\cdot(dAdT)_9\) data and previously reported TRIR data on poly(dAdT)\(^6\) collected using a flow cell suggests that minimal melting of the duplexes is occurring using this method.

2.4. Steady-State Measurements

2.4.1. **UV/Vis Absorption Spectra**

Steady-state UV/Vis absorption spectra were measured on a Lambda 25 spectrometer (Perkin-Elmer, Wellesley, MA) and a Perkin-Elmer 20 (Perkin-Elmer, Wellesley, MA) at room temperature. Identical spectra were obtained for both spectrometers. Samples were held between CaF\(_2\) windows in a home-built sample cell with a 1.2 mm Gylon spacer for UV- and visible-probe experiments and a 100-\(\mu\)m PTFE spacer for mid-IR experiments.

2.4.2. **Circular Dichroism Spectra**

Steady-state circular dichroism (CD) spectra were recorded on an Aviv (Pistacaway, NJ) Model 202 circular dichroism spectropolarimeter. For room temperature spectra, samples were held in 1-mm pathlength quartz spectrophotometer cells (Starna Cells, Inc., Atascadero, CA). Reported spectra are the average of 3 scans and a
background solvent spectrum was subtracted from the sample spectrum. For melting curve experiments, samples were held in 1-cm pathlength quartz spectrophotometer cells. The CD spectra were recorded every 10°C from 20 to 80°C after a 5-minute equilibration period. Melting curves were generated by converting the CD dynode signals to absorption spectra using a standard routine in the Aviv software and plotting the absorption values at 260 nm as a function of temperature. The absorption values were normalized to give the maximum absorption value a value of 1.

2.4.3. FTIR Spectra.

Steady-state FTIR spectra were recorded on a Perkin-Elmer Spectrum 2000 FTIR spectrometer at room temperature. Samples were held between two CaF$_2$ windows or a BaF$_2$/CaF$_2$ window combination in a home-built sample cell with a 100-µm PTFE spacer. A solvent background spectrum was manually subtracted from the sample spectrum.

2.5. Data Treatment

Transient signals were globally fit to sums of exponentials convoluted with a Gaussian instrument response function using the global fitting routine included in IGOR Pro versions 5.04 and 6.21 (WaveMetrics, Inc., Portland, OR). For UV probe transients, the instrument response was determined by fitting the coherent two-photon signal of a solvent-only scan. For visible probe transients, the instrument response time was included as a fit parameter. Typical values for the instrument response were 250 fs for the UV probe and 350 fs for the visible probe. All uncertainties reported are 2σ.
Solvated electrons created by two-photon ionization of the solvent absorb at 570 nm, making it necessary to do a background subtraction. The method for correcting for two-photon ionization signals was described by Crespo-Hernández et al.\textsuperscript{7} Briefly, equimolar monomer samples were prepared to have an absorbance of 1 at the pump wavelength. The monomer samples have a known ultrafast decay such that any signal at long times comes from solvated electrons. A solvent-only background scan was scaled at long times to the monomer signals. This scaled solvent-only background was subtracted from oligonucleotide signals at 570 nm. All transient signals reported at 570 nm have been corrected for solvated electron signals.

2.6. Akaike Information Criterion

A challenge with fitting multiexponential transient absorption signals is selecting the best fitting model.\textsuperscript{8} Numerous mathematical strategies have been developed to help determine the best-fit model for multiexponential kinetic data. One such strategy is the Akaike Information Criterion (AIC)

\[
AIC = N \ln \left( \frac{SS}{N} \right) + 2(K + 1)
\]

where SS is the sum of squared deviation, N is the number of data points and K is the number of estimated parameters.\textsuperscript{9} With this strategy, the fitting model with the minimum AIC value is considered the best model for the data. For small sample sizes, where $N/K < 40$, a modified version of the AIC
called the corrected Akaike Information Criterion (AICc) has been shown to be better at avoiding the overfitting data. In Chapters 4, 5, and 6, the AICc strategy was used to determine the best fitting model for the transient absorption signals.

\[ AIC_c = N \ln \left( \frac{SS}{N} \right) + 2(K + 1) + \frac{2(K + 1)(K + 2)}{N - K - 2} \] 

References


Figure 2.1. Experimental layout for the UV-pump / UV- and visible-probe transient absorption spectrometer.
Figure 2.2. Experimental layout for the UV-pump / mid-IR probe transient absorption spectrometer.
Figure 2.3. Comparison of FTIR spectra of DNA samples obtained from different sources. TMP and poly(A) were obtained from Sigma-Aldrich as sodium salt forms. (dAdT)$_9$ was obtained from Midland as ammonium and sodium salt forms. The intense band at 1674 cm$^{-1}$ is present only in the ammonium salt form of (dAdT)$_9$. 
Figure 2.4. Mid-IR transient absorption spectra of (dA)$_{18}$ in unbuffer D$_2$O at select time delays following 270 nm excitation.
Figure 2.5. Schematic of IR spinning cell.

IR spinning cell

a) Top view: cap
b) Cap
c) Insert
d) Vitron o-ring
   1 in OD, 1/16” width
e) BaF2 VUV window
   Korth Kristalle GmbH
   25 mm dia, pathlength 2 mm
f) PTFE spacer
   Sigma-Aldrich
   25 mm dia, pathlength 0.1 mm
g) BaF2 VUV window
h) Cell base
i) Top view: cell base

1.50 inches
Figure 2.6. Consecutive screen shots of (dA)$_{18}$-(dT)$_{18}$ spectrum at a time delay of 1 ps after a) 12 s, b) 24 s, c) 27 s and d) 40 s of exposure to 271 nm pump. The sample holder was held stationary while the sample cell was spun at several hundred rpm. The red curve is the 1 ps trace after prolonged (>5 min) exposure.
Figure 2.7. FTIR spectra of \((dA)_{18}\), \((dT)_{18}\), and the duplex \((dA)_{18} \cdot (dT)_{18}\) in phosphate D$_2$O buffer.
Chapter 3: Excited-State Dynamics of GC-containing DNA

3.1. Introduction

The iconic double-helical structure first described by Watson and Crick in 1953 is just one of many possible DNA structures.\(^1\) DNA can form into a variety of stacking and pairing arrangements: ranging from the well-known B-form duplex to G-quadruplexes and hairpins. The first reported X-ray structure of DNA was actually of the left-handed Z-form duplex.\(^2\) Sequence and solvent conditions strongly influence the secondary structure of DNA, affecting the base stacking and pairing geometries. One of our interests is in the effect of base pairing and base stacking interactions on the excited-state dynamics of DNA. The importance of base stacking to long-lived excited states has been illustrated in A- and T-containing DNAs;\(^3\) however, less is know about how base stacking and pairing affect the dynamics in other DNA systems.

The results of femtosecond fluorescence up-conversion studies of GC-DNA have lead to questions of whether long-lived excited states like those in AT-containing DNA are also present in GC-containing DNA.\(^4\)\(^-\)\(^6\) Miannay \textit{et al.} reported subpicosecond emissive decay from poly(dGdC)·poly(dGdC). An average lifetime of 200 fs was reported for the duplex poly(dGdC)·poly(dGdC) at 330 nm, while average lifetimes of 450 and 340 fs were reported for the monomers dCMP and dGMP, respectively.\(^4\) Single
GC base pairs, made from modified G and C bases, were reported to have a fluorescence lifetime of 0.355 ps in chloroform.\textsuperscript{5,6} The individual modified G and C bases had longer lifetimes of 0.84 and 0.67 ps, respectively. It has been proposed that proton transfer in Watson-Crick (WC) GC base pairs can lead to excited-state quenching.\textsuperscript{7-9} However, the absence of long-lived states in these studies does not mean that they are not present. The long-lived states observed in AT-DNA are dark states and the radiative decay rate of these states are too low to be detected by fluorescence up-conversion spectroscopy.

To determine whether long-lived states are present in GC-containing DNA, transient absorption experiments were carried out on a series of alternating and non-alternating GC-containing oligonucleotides.\textsuperscript{10,11,12} Long-lived excited states are observed in high yield in all GC-containing oligonucleotides studied.\textsuperscript{12} The lifetimes of the long-lived excited states are sensitive to the base sequence as will be shown in this chapter.

The effects of base-stacking and base-pairing on the excited-state dynamics of the self-complementary oligonucleotide d(GC)<sub>9</sub>·d(GC)<sub>9</sub> were also studied. This unique DNA duplex undergoes significant conformational changes in solutions of different ionic strength and pH, allowing the effect of base overlaps on excited-state dynamics to be studied in a system with constant base sequence. In low salt conditions, d(GC)<sub>9</sub>·d(GC)<sub>9</sub> adopts the typical B-conformation, but in high salt conditions, the sequence undergoes a structural transition to the left-handed Z-conformation shown in Figure 3.1. At low pH, protonation of cytosine induces a change in base pairing motif within the duplex from Watson-Crick to Hoogsteen (Figure 3.1). Transient absorption measurements were carried out on d(GC)<sub>9</sub>·d(GC)<sub>9</sub> under the different solvent conditions and the excited-state
dynamics were found to be nearly independent of base stacking geometries and base pairing motifs.

Finally, alternating and non-alternating sequences were studied in H₂O and D₂O to investigate the role of interstrand processes in the excited-state dynamics. It has been proposed the formation of intrastrand excited states with significant charge transfer character can trigger interstrand proton transfer. A pronounced deuterium isotope effect is reported for alternating GC-DNA but not for the non-alternating sequence. This result is consistent with the greater charge transfer character expected for a GC exciplex state compared to a GG or CC excimer state and parallels the results reported for AT-DNAs. Blocking base pairing through base modification, a single-stranded analog to the alternating GC-DNA was studied and no isotope effect was observed, supporting the involvement of proton transfer in alternating GC-DNA duplexes.

3.2. Results

3.2.1. Transient Absorption Measurements of GC-containing Oligonucleotides

Transient absorption signals for the GC-containing oligonucleotides d(GC)_9·d(GC)_9, d(C₄G₄), d(C₅T₄G₅) and d(C₅A₄G₅) recorded at probe wavelengths of 570 and 250 nm following excitation at 266 nm are shown in Figure 3.2. For comparison, the transient signals of the equimolar mixture of the constituent bases GMP and CMP are also shown. The positive signals at 570 nm monitor excited-state relaxation, while bleach signals at 250 nm probe ground-state repopulation. It is clear in Figure 3.2 that the GC-oligonucleotides signals have a slower decay compared to the monomer
mixture. The monomer signals at 570 nm decay with a lifetime of 0.6 ± 0.1 ps. At 250 nm, the monomers recovery is well fit to two exponentials, with lifetimes of 2.0 ± 0.3 and 31 ± 26 ps. The 2 ps lifetime of the ground state recovery of the fast component matches the characteristic lifetime for vibrational cooling in monomer bases. The longer-lived component likely results from aggregation of GMP in solution as it is also observed in pure GMP solutions and not the \(^1\text{n}\pi^*\) state of cytosine, as originally assigned in ref. 12. GMP has been shown to self-aggregate into stacked monomers and tetramers in neutral solution.  

Measurements on single-stranded oligodeoxynucleotides composed solely of cytosine and guanine bases are not feasible due to the formation of higher-order structures. The 18-mer d(C)\textsubscript{18} has been shown to form a hemi-protonated duplex in neutral and near-neutral pH values because of its high pKa value, \(^18\text{-}21\) and d(G)\textsubscript{18} readily self-associates to form a quadruplex structure in neutral phosphate buffer. \(^22,23\) The stability of these higher-order structures resulted in unsuccessful attempts to form the (dG)\textsubscript{18}′(dC)\textsubscript{18} duplex as determined by CD measurements. In order to study the excited-state dynamics of non-alternating GC sequences, d(C\textsubscript{4}G\textsubscript{4}), d(C\textsubscript{5}T\textsubscript{4}G\textsubscript{5}) and d(C\textsubscript{5}A\textsubscript{4}G\textsubscript{5}), which have regions of consecutive G and C bases, were used. The sequences d(C\textsubscript{5}T\textsubscript{4}G\textsubscript{5}) and d(C\textsubscript{5}A\textsubscript{4}G\textsubscript{5}) form hairpins with B-form stacking in the stem region and poorly stacked loop regions. \(^24\) The octamer d(C\textsubscript{4}G\textsubscript{4}) is self-complementary and forms a duplex with A-like stacking with B-type sugar puckering. \(^25\)
Signals from the oligonucleotides with consecutive tracts of guanines and cytosines (referred to as G- and C-tracts) exhibit biexponential decays. Hairpin forming d(C₅T₄G₅) and d(C₅A₄G₅) have nearly identical decays with lifetimes of 1.1 ± 0.2 and 3.0 ± 0.4 ps for the fast component at 570 nm and 250 nm, respectively, and 41 ± 6 ps for the long-lived state. The self-complementary d(C₄G₄)·d(C₄G₄) has lifetimes of 1.3 ± 0.3 and 3.3 ± 0.6 ps for the fast component at 570 nm and 250 nm, respectively, and 22 ± 6 ps for the long-lived component. In contrast, the alternating d(GC)₉·d(GC)₉ has a single exponential decay with lifetime that is faster than the slow components, but also slower than the fast component in the non-alternating sequences. The signals at 570 and 250 nm have lifetimes of 4.1 ± 0.3 and 6.3 ± 0.6 ps, respectively. Fit parameters are given in Table 3.1.

3.2.2. Steady-State Absorption and CD spectra of d(GC)₉·d(GC)₉

Steady-state absorption and CD spectra of d(GC)₉·d(GC)₉ in low salt (0.25 M), high salt (5 M), and low pH (3.6) conditions are shown in Figure 3.3. The absorption spectrum of d(GC)₉·d(GC)₉ in high salt conditions has a decreased intensity at 255 nm and is red shifted compared to the low salt conditions. In acidic conditions, a decreased intensity at 255 nm and an increased intensity at 285 nm are observed.

Strikingly different CD spectra are observed for the different solvent conditions. In low salt conditions, the CD spectrum of d(GC)₉·d(GC)₉ has a positive band at 280 nm and a negative band at 250 nm. In high salt conditions, a near inversion of the low salt conditions spectrum is observed with a minimum at 293 nm and a maximum at 275 nm.
The CD spectrum of d(GC)<sub>9</sub>·d(GC)<sub>9</sub> in low pH is similar to low salt conditions, but with broader bands and a maximum at 290 nm and a minimum at 250 nm.

3.2.3. Transient Absorption Measurements of d(GC)<sub>9</sub>·d(GC)<sub>9</sub> in Conditions of High Ionic Strength and Low Acidity

Despite differences in base stacking geometries between the two conformations, nearly identical ground-state bleach signals (266 pump / 250 nm probe) are observed for B- and Z-forms in Figure 3.4a. Signals for B- and Z-forms d(GC)<sub>9</sub>·d(GC)<sub>9</sub> recover with a lifetimes of 6.3 ± 0.3 ps and 7.5 ± 0.3 ps, respectively. Fig. 3.4b compares signals for d(GC)<sub>9</sub>·d(GC)<sub>9</sub> with Watson-Crick and Hoogsteen base pairing. The d(GC)<sub>9</sub>·d(GC)<sub>9</sub> has a slightly faster recovery with a lifetime of 5.3 ± 0.6 ps. Fit parameters are given in Table 3.2.

3.2.4. Transient Absorption Measurements of d(GC)<sub>9</sub>·d(GC)<sub>9</sub> in H<sub>2</sub>O and D<sub>2</sub>O

Figure 3.6 shows back-to-back transient absorption signals of d(GC)<sub>9</sub>·d(GC)<sub>9</sub> in H<sub>2</sub>O and D<sub>2</sub>O at a pump wavelength of 266 nm and a probe wavelength of 250 nm. In H<sub>2</sub>O, the signal recovers with a single lifetime of 6.3 ± 0.3 ps. In D<sub>2</sub>O, an additional decay of 22 ± 4 ps component appears. The relative amplitude of this component is 58%. The CD spectra of d(GC)<sub>9</sub>·d(GC)<sub>9</sub> in H<sub>2</sub>O and D<sub>2</sub>O are shown in Figure 3.5. No significant difference in CD signal is observed between the two solvents.

In contrast, no difference in transient absorption signals is observed for d(C<sub>4</sub>G<sub>4</sub>)·d(C<sub>4</sub>G<sub>4</sub>) in H<sub>2</sub>O and D<sub>2</sub>O (Figure 3.6). In order to investigate the excited-state dynamics of an alternating GC system in the absence of base-pairing interactions, cytosine bases were modified by the addition of a bulky methyl group at the N3 position. This
modification has been shown to hinder base pairing between cytosine and guanine.\textsuperscript{26} Signals from the modified single-stranded alternating d[(GX)\textsubscript{9}GC], where X is 3-methylcytidine, are shown in Figure 3.6. A small kinetic isotope effect is observed, but to a lesser degree than in the unmodified duplex. The signals are well fit to two exponentials plus an offset, with a long-lived component with a lifetime of 25 ± 5 ps and a fast component of 5.5 ± 1.0 ps in H\textsubscript{2}O which slows to 6.6 ± 2.0 ps in D\textsubscript{2}O. Lifetimes and amplitudes are given in Table 3.3.

3.3. Discussion

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

Long-lived excited states with lifetimes at least an order of magnitude longer than the monomer constituents are observed in high yield for all the GC-containing oligonucleotides studied. The near-mirror symmetry seen in Figure 3.2 between 570 nm and 250 nm, with the exception of the monomer mixture, indicates that the excited states monitored at 570 nm decay back to the ground state and additional pathways (ie. \textsuperscript{1}n\pi* or \textsuperscript{3}π\pi*) are not present.\textsuperscript{27,28} This result shows that long-lived excited states similar to those observed in in A- and T- containing DNA\textsuperscript{3,29-31} are also present in GC-containing DNA. These states are assigned to exciplex states formed between a pair of stacked bases.\textsuperscript{3,13}

The detection of long-lived excited states in GC DNA provides a different picture to femtosecond fluorescence up-conversion experiments, which show emission on the time scale of hundreds of femtoseconds.\textsuperscript{4,5} This difference is explained by the "dark"
character of the long-lived excited states. The low radiative transition probability of these states make them difficult to detect using fluorescence up-conversion spectroscopy, which monitors excited-state emission; whereas, they are readily observed in excited-state absorption and ground-state bleach signals. Recent studies by Markovitsi and coworkers using the more sensitive technique of time-correlated single photon counting (TCSPC) have detected emission out to nanosecond time scales in poly(dGdC)·poly(dGdC) and poly(dG)·poly(dC). The connection between these long-lived ns emissive states and the long-lived excited states reported in this study is a topic of future interest. The model for the excited-state dynamics in DNA oligonucleotides that has been proposed based on the combined results of transient absorption and fluorescence up-conversion studies involves the initial formation of excitonic states, delocalized over a few bases that localize in less than a picosecond to exciplex states formed between a pair of bases.

Although the DNA oligonucleotides are all composed of guanine and cytosine bases, significantly different transient absorption signals are observed in Figure 3.2. The long-lived decay signals are sensitive to base sequence. The alternating d(GC)$_9$·d(GC)$_9$ has an excited-state lifetime of 6 ps, while the non-alternating d(C$_4$G$_4$)·d(C$_4$G$_4$) and hairpins have longer lifetimes of 22 and 44 ps, respectively. DNA exciplex lifetimes have been shown to decrease with increasing thermodynamic driving force for charge recombination in minimal base stacks. The shorter lifetime for d(GC)$_9$·d(GC)$_9$ is consistent with the lower energy for the G$^+$C$^-$ state compared to G$^+$G$^-$ and C$^+$C$^-$ states and supports the assignment of these states to exciplexes.
The alternating d(GC)$_9$·d(GC)$_9$ transient absorption signals in Figure 3.2 are well fit to a single exponential while the other non-alternating GC systems have a biexponential decay with a fast 2 ps component observed at 250 nm. Similar biexponential decays have also been observed in AT-DNAs, with the fast component assigned to monomer-like decay in unstacked bases and the long-lived decay component assigned to excimer states.$^{13}$ The absence of a 2 ps component at 250 nm and the subpicosecond component at 570 nm in the transient signals of in d(GC)$_9$·d(GC)$_9$ suggests that there is little ultrafast internal conversion back to the ground state in this system. This sequence has a high melting temperature and a greater degree of base stacking is expected compared to the other GC-containing systems. It is also expected to have more stacking than AT-containing systems because terminal GC base pairs have less end fraying than AT base pairs.$^{35,36}$ This result supports that π-stacking leads to long-lived excited states and that branching to the ground state is not present in this system.

### 3.3.2. The Excited-State Lifetimes in a G·C DNA Duplex are Nearly Independent of Helix Conformation and Base-Pairing Motif

Steady-state absorption and CD spectra of d(GC)$_9$·d(GC)$_9$ in Figure 3.3 provide structural information about the duplex under different solvent conditions. The CD spectrum of d(GC)$_9$·d(GC)$_9$ in neutral, low salt (0.25 M) solution has the characteristic shape of B-form DNA. In high (5 M) salt conditions, the inversion of the CD spectrum relative to low salt, indicates that the duplex has undergone a structural transition from a B-form to a left-handed Z-form structure (Figure 3.1).$^{37}$ The low (3.6) pH absorption spectrum has an increase in the absorbance at 285 nm that is assigned to protonation of
cytosine bases.\textsuperscript{38} The protonation of cytosine induces a change in base pairing motif from Watson-Crick to Hoogsteen within the duplex.\textsuperscript{38,39} The base pairing motifs for Watson-Crick and Hoogsteen base pairing are shown in Figure 3.1. Antao et al. proposed that at pH 3.0, approximately 50\% of the base pairs were protonated.\textsuperscript{39} The CD and absorption spectrum are characteristic of Hoogsteen base pairing, although the precise duplex conformation is unknown.\textsuperscript{38} The combination of CD and absorption spectra confirm that we generated different stacking conformations and base pairing motifs under our experimental conditions.

Despite significant differences in base stacking geometries between the two conformations, illustrated in Figure 3.1, nearly identical signals are observed for B- and Z-forms of d(GC)\textsubscript{9}·d(GC)\textsubscript{9} (Figure 3.4). The Z-form has slightly slower decay of 6.3 ± 0.6 ps vs. 7.6 ± 0.8 ps for the B-form in H\textsubscript{2}O, but this is only a 20\% slowing of the signal. Crespo-Hernández et al. studied the effect of conformation on the electronic excited-state dynamics of two single-stranded A-containing systems: poly(A) with RNA-like A-form stacking and poly(dA) with DNA-like B-form stacking.\textsuperscript{29} Similar results were reported, with identical decay lifetimes observed for both systems and differences only observed in the amplitudes of the different decay components.

Although the DNA backbone is rather flexible, it is extremely unlikely that the same excited-state geometry could be obtained in all duplex forms within the several picosecond lifetime of the exciplex. Assuming that no large structural changes occur upon excitation, which is reasonable given the short excited-state lifetime for this system,
the 5'-CpG-3' and 5'-GpC-3' steps have significantly different base stacking geometries in B- and Z-conformations as shown in Figure 3.1.

One possible explanation for the similar excited-state dynamics for B- and Z-conformations is that processes occurring within the WC base pairing, which is conserved in both forms, play a dominant role in the excited-state dynamics. To rule out the possibility that the similar signals result from interstrand processes, measurements were carried out on d(GC)$_9$$\cdot$$d$(GC)$_9$ at low pH where the WC base pairs become protonated and form Hoogsteen base pairs (Figure 3.1). Nearly identical decay signals are observed for d(GC)$_9$$\cdot$$d$(GC)$_9$ with WC base pairing and Hoogsteen base pairing (Figure 3.4). This result shows that interstrand processes are not responsible for the similar excited-state dynamics in B- and Z-forms of d(GC)$_9$$\cdot$$d$(GC)$_9$. Ultrafast decay have been proposed to occur in WC base pairs, but not non-WC base pairs, in gas-phase studies$^7$ and proposed to result from electron-coupled proton transfer.$^9$ The similar bleach signals for different base pairing also indicates that there is no unique rapid decay pathway for the WC base pairs compared to Hoogsteen in d(GC)$_9$$\cdot$$d$(GC)$_9$. Although no difference is observed in the decay signals for these two systems, that does not mean that interstrand processes do not contribute to the excited-state dynamics in this stacked duplex. In Section 3.3.3, evidence for interstrand processes in DNA will be discussed.

The similar decays for B- and Z-conformations are also not explained if discussed in terms of the long-lived states to excitons$^{31,41}$ rather than exciplexes. It is reasonable to expect different dynamics from excitons in these systems as well. Differences in the UV absorption spectra of the various duplexes (Figure 3.3) indicate that each duplex has a
unique set of excitonic states. If the long-time decays are from excitonic states, it is
difficult to reason why changes to these states would not also produce changes in the
decay times.

Hole transfer (HT) and excess electron transfer (EET) in DNA have been shown
to be sensitive to conformation. Experimental studies have shown that the rate of excess
charge transport in DNA is affected by base stacking alignment. Experimental studies
predict that changes in conformation significantly alter charge-migration and electronic
coupling for HT and EET. It is at first surprising that similar exciplex lifetimes are
observed for the B- and Z-conformations; however, there are important differences
between HT and EET and exciplex decay in DNA. HT and EET involve donor and
acceptor molecules separated by at least a base. Electron transfer between these
molecules is proposed to occur in the nonadiabatic limit with the rate varying as the
square of the electronic coupling. In the case of exciplex decay, charge recombination
occurs between a pair of radical ions on adjacent bases. The electronic coupling for
charge recombination in this system is expected to be greater than HT or EET due to
larger energy splitting between the adiabatic states. We propose that electronic coupling
for exciplex decay is large and insensitive to the base conformations. The nonadiabatic
electron transfer description may fail to describe charge recombination in these states due
to the large electronic coupling.

Rather than depending strongly on electronic coupling for charge recombination,
the exciplex lifetimes is proposed to depend strongly on ΔG. Takaya et al. showed that
exciplex lifetimes were related to the thermodynamic driving force for charge
recombination in a series of dinucleosides. The driving force is estimated as the difference between the gas-phase ionization energy (IP) of the electron donor base and the gas-phase electron affinity (EA) of the acceptor base. Calculations of the ionization energy for GG/CC base pairs show a change of only about 8% between B- and Z-conformations. If similar results are obtained for the electron affinity, then the driving force for charge recombination would be similar for B- and Z-conformations and the exciplex lifetimes would also be similar as is observed in our UV signals.

The B- and Z-forms of poly(dGdC)·poly(dGdC) were studied using ps-TRIR spectroscopy by George and co-workers. A biexponential decay was reported for the vibrational bleach signals for the B-conformation, while the bleach signals for the Z-form were fit to a single exponential. This difference in vibrational signals is puzzling given the similar UV signals in H$_2$O shown in Figure 3.4. Although different dynamics are reported for these two conformations, both systems relax on a similar time scale. The slower decays reported for the mid-IR bleach signals of B- and Z-forms of poly(dGdC)·poly(dGdC) in D$_2$O compared to our UV bleach signals in H$_2$O are shown to result from differences in solvents (H$_2$O vs. D$_2$O) and will be discussed in Section 3.3.3.

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

Annealing the DNA samples in D$_2$O, as done in these experiments, disrupts base pairing and stacking interactions and leads to the exposure of bases normally held within the duplex to the surrounding solvent molecules. Lane and Thomas found that exposing 5'-GMP to D$_2$O resulted in the immediate isotope substitution of the N1 and
Under prolonged exposure, isotope substitution also occurred at the C8 hydrogen. In our experimental conditions, we expect to have isotopic substitution of the N1 and N2 hydrogens of guanine.

A pronounced deuterium kinetic isotope effect is observed on the excited-state decays of alternating d(GC)$_9$·d(GC)$_9$ in D$_2$O. In D$_2$O, the initial decay component remains the same, but a long-lived 22 ± 6 ps component that accounts for approximately half of the amplitude of the ground-state bleach appears that is not seen for the same duplex in H$_2$O. An isotope effect is not observed in the non-alternating d(C$_4$G$_4$)·d(C$_4$G$_4$) signals (Figure 3.6) and only a modest isotope effect is observed in the signal for the equimolar mixture.

These results follow the same trend previously reported for AT DNAs: An isotope effect is observed in the alternating sequence, but not in its non-alternating analogue. A lengthening in the lifetime of the long-lived component in D$_2$O by a factor of 2.3 was previously reported by Crespo-Hernandez et al. in alternating d(AT)$_9$·d(AT)$_9$, but not in the non-alternating duplex d(A)$_{18}$·d(T)$_{18}$. It was proposed that only exciplex states with significant charge transfer character can undergo interstrand proton transfer. The observed isotope effect in d(GC)$_9$·d(GC)$_9$, but not in d(C$_4$G$_4$)·d(C$_4$G$_4$), is consistent with the expected strong CT character of a GC-stacked exciplex due to their differences in redox potentials.

The ideal way to determine whether base-pairing interactions contribute to the excited state dynamics is to remove those interactions from the system. However, the self-complementary nature of alternating d(GC)$_9$·d(GC)$_9$ makes it difficult to isolate and
study base stacking effects in the absence of base pairing. The modified d[(GX)_9GC],
where X is 3-methylcytidine, has a bulky methyl group that hinder base pairing and
allows us to probe the dynamics in a stacked but unpaired system. As shown in Figure
3.6, only a modest kinetic isotope effect is observed for single-stranded d[(GX)_9GC] in
D_2O. The observed kinetic isotope effect is similar to that seen in the equimolar solution
and results from different rates of vibrational cooling in H_2O and D_2O following ultrafast
internal conversion to the ground state. The observation of an isotope effect in the
alternating double-stranded d(GC)_9·d(GC)_9, but not in the modified single-stranded
d[(GX)_9GC] where base pairing is not present, supports that interstrand processes
contribute to the excited-state dynamics in duplex DNA. The kinetic isotope effect seen
alternating GC and AT duplexes, but not in the non-alternating analogs which have
identical base pairing motifs indicates that the observed isotope effect is related to more
than just base pairing. The combined results for these different systems support the
concept that long-lived exciplex states with significant CT character can decay via a
mechanism involving interstrand proton transfer.

Theoretical studies provide insight into the feasibility of proton transfer in these
systems. The barriers to proton transfer are calculated to be lower for both the one-
electron oxidized and one-electron reduced GC base pairs than for neutral pairs. A
modest activation barrier was predicted for proton transfer in the GC radical cation, but
proton transfer was predicted to be energetically favorable for the GC radical anion base
pair, with a free-energy change of -3 kcal/mol and a small activation barrier. Bertran et
al. predicted that proton transfer was an endergonic reaction for the GC radical cation
base pair, with a calculated barrier of 4.3 kcal/mol. Recent work by Kumar and Sevilla found that including water molecules to mimic the hydration environment in DNA made proton transfer for the GC radical cation pair become favorable.\textsuperscript{56} The free energy change was predicted to be -0.65 kcal/mol with an activation energy of 1.42 kcal/mol. These studies support that interstrand proton transfer may occur following formation of an exciplex state with significant charge transfer character as will discussed further in Chapter 5.

3.4. Conclusions

Long-lived excited states are shown to form in high yields in GC-containing oligonucleotides, extending the previous results for AT DNA.\textsuperscript{3,12,29} Transient absorption experiments on alternating d(GC)$_9$·d(GC)$_9$ in conditions that promote different structural conformations reveal that these long-lived states are insensitive to changes in base-stacking geometries and base-pairing motifs,\textsuperscript{10} while sequence context plays a significant role in the excited-state dynamics. Furthermore, a pronounced isotope effect observed for transient signals of d(GC)$_9$·d(GC)$_9$ in D$_2$O, but not in signals for non-alternating d(C$_4$G$_4$) or single-stranded d[(GX)$_9$GC], suggests that interstrand interactions play a role in the excited-state dynamics.\textsuperscript{11} The role of proton transfer in the excited-state dynamics of DNA is discussed further in Chapter 5.
References


Figure 3.1. a) Base–base overlaps for B- and Z-conformations viewed along the helix axis and side profiles. b) Watson–Crick and Hoogsteen G·C base pairing motifs. Duplex structures and B-form overlap structures were generated in Hyperchem (Hypercube, Inc., Gainesville, Fl). Base overlap structures for Z-form were obtained from X-ray diffraction studies (PDB: 1DCG). Structures were visualized using VMD and Chimera software. Figure adapted from Ref. 34.
Figure 3.2. Normalized transient absorption signals for various GC-containing oligonucleotides and an equimolar mixture of CMP and GMP probed at 570 nm and 250 nm following 266 nm excitation. Best-fit curves are shown by solid lines. Figure adapted from Ref. 12.
Figure 3.3. Steady-state absorption and CD spectra of d(GC)$_9$ · d(GC)$_9$ in low salt (0.25 M), high salt (5 M), and low pH (3.6) conditions.
Figure 3.4. Normalized bleach signals (266 nm pump/250 nm probe) for d(GC)$_9$·d(GC)$_9$ in a) B- and Z-conformation and b) Watson-Crick and Hoogsteen base pairing. Best-fit curves are shown by solid lines.
Figure 3.5. CD spectra of d(GC)$_9$·d(GC)$_9$ in H$_2$O and D$_2$O.
Figure 3.6 Normalized bleach signals (266 nm pump / 250 nm probe) for a) equimolar mixture of CMP + GMP, b) d(GC)$_9$·d(GC)$_9$, c) d(C$_4$G$_4$)·d(C$_4$G$_4$), and d) single-stranded d[(GC)$_9$GC] in H$_2$O (open circles) and D$_2$O (closed circles). Best-fit curves are shown by solid lines.
Table 3.1. Best-fit lifetimes and relative amplitudes obtained from global fits of transient absorption signals for various G·C duplexes and an equimolar mixture of CMP and GMP (shown in Figure 3.1). All uncertainties are twice the standard error.

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<th>(A_2) (%)</th>
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<td>(\text{a})</td>
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<td>(\text{a})</td>
<td>6 ± 1</td>
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\(^a\)Globally linked to value in preceding line of the table.

\(^b\)Globally linked to corresponding value for \(d(C_5T_4G_5)\).
Table 3.2. Best-fit parameters for transient absorption signals (266 nm pump / 250 nm probe) of d(GC)$_9$·d(GC)$_9$ shown in Figure 3.4. All uncertainties are twice the standard error.

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<th>$\tau_1$ (ps)</th>
<th>$A_2$ (%)</th>
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<td>6.4 ± 0.6</td>
<td>2 ± 2</td>
</tr>
<tr>
<td></td>
<td>570</td>
<td>100</td>
<td>3.6 ± 1.0</td>
<td>--</td>
</tr>
<tr>
<td>Z-form$^b$</td>
<td>250</td>
<td>98 ± 4</td>
<td>7.6 ± 0.8</td>
<td>2 ± 2</td>
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<tr>
<td></td>
<td>570</td>
<td>100</td>
<td>4.8 ± 0.4</td>
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</tr>
<tr>
<td>Hoogsteen$^c$</td>
<td>250</td>
<td>96 ± 4</td>
<td>5.3 ± 0.6</td>
<td>4 ± 1</td>
</tr>
</tbody>
</table>

$^a$pH 6.8, 0.25 M NaCl
$^b$pH 6.8, 5.0 M NaCl
$^c$pH 3.0, 0.25 M NaCl
Table 3.3. Lifetimes and relative amplitudes (in parentheses) from global fits to transient signals shown in Figure 3.6. Amplitudes do not sum to one hundred percent due to a small amount of residual photobleaching. Identical values in the same column were linked during global fitting. All uncertainties are twice the standard error.

<table>
<thead>
<tr>
<th>System</th>
<th>Solvent</th>
<th>$\tau_1$ (ps)</th>
<th>$\tau_2$ (ps)</th>
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<tr>
<td>CMP + GMP</td>
<td>H$_2$O</td>
<td>2.0 ± 0.3 (92)</td>
<td>31 ± 26 (8)</td>
</tr>
<tr>
<td></td>
<td>D$_2$O</td>
<td>2.6 ± 0.4 (91)</td>
<td>31 ± 26 (9)</td>
</tr>
<tr>
<td>d(GC)$_9$·d(GC)$_9$</td>
<td>H$_2$O</td>
<td>6.3 ± 0.4 (98)</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>D$_2$O</td>
<td>6.3 ± 0.4 (41)</td>
<td>22 ± 4 (56)</td>
</tr>
<tr>
<td>d(C$_4$G$_4$)·d(C$_4$G$_4$)</td>
<td>H$_2$O</td>
<td>3.1 ± 0.7 (61)</td>
<td>22 ± 5 (32)</td>
</tr>
<tr>
<td></td>
<td>D$_2$O</td>
<td>3.1 ± 0.7 (62)</td>
<td>22 ± 5 (33)</td>
</tr>
<tr>
<td>d(IC)$_9$·d(IC)$_9$</td>
<td>H$_2$O</td>
<td>0.70 ± 0.08 (82)</td>
<td>48 ± 18 (18)</td>
</tr>
<tr>
<td></td>
<td>D$_2$O</td>
<td>0.70 ± 0.08 (82)</td>
<td>48 ± 18 (18)</td>
</tr>
<tr>
<td>d[(GX)$_9$GC]</td>
<td>H$_2$O</td>
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<td>D$_2$O</td>
<td>6.6 ± 2.0 (55)</td>
<td>22 ± 5 (43)</td>
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</table>
Chapter 4: Observation of Long-Lived States in DNA Oligonucleotides with Significant Sequence Disorder

4.1. Introduction

An experimental approach used in the study of the excited-state dynamics of DNA has been to start with the building blocks and work up in structural complexity. Time-resolved studies on the individual natural DNA bases revealed that ultrafast deactivation channels are present that lead to decay back to the ground state in less than a picosecond in the purine bases and that additional long-lived $^3\pi\pi^*$ and $^1\pi\pi^*$ states are present in the pyrimidine bases. Increasing the structural complexity, studies were carried out on single-stranded multimers composed entirely of adenine bases. These systems were reported to decay on two time scales: an ultrafast 2 ps decay assigned to unstacked bases and a long-lived ~100 ps component assigned to excimer states formed between stacked bases. Adding the complementary thymine strand to form a duplex introduced base pairing interactions, but did not significantly change excited-state dynamics. Long-lived excited-states have since been observed in model systems ranging from the most basic stacked systems to single- and double-stranded systems composed of alternating and non-alternating AT\textsuperscript{2,3,5-7} and GC bases.

Although much progress has been made in the area of DNA photophysics, many questions remain including whether the long-lived states observed in model DNA
systems are still present in mixed-sequence DNA. It has been suggested that the long-lived excited states observed in well-ordered model systems may be absent in more disordered sequences common in genomic DNA.\textsuperscript{7,13} Schwalb and Temps systematically varied base sequences in strands composed purely of purine or pyrimidine bases.\textsuperscript{13} Monitoring excited-state emission signals, they noted that the amplitude of the long-lived component was negligible in systems with increased sequence disorder. Buchvarov \textit{et al.} suggested that systems with significant sequence disorder might not have long-lived excited states, rather sequence disorder may result in localized monomer-like states that return excess electronic energy to the ground state via ultrafast internal conversion.\textsuperscript{7} The absence of long-lived states in genomic DNA would have important photochemistry implications.

To investigate the effect of sequence disorder on the excited-state dynamics in single-stranded DNA and how the excited-state dynamics change when base pairing is present, the \textit{ras61} oligodeoxynucleotide sequence was studied by transient absorption spectroscopy.\textsuperscript{14,15} The \textit{ras61} oligodeoxynucleotide consists of a purine-rich d(CGGACCAAGAAG) strand (hereafter referred to as strand A) and its complementary pyrimidine-rich d(CTTCTTGTCGG) strand (hereafter referred to as strand B). The \textit{ras61} oligodeoxynucleotide is of biological interest because it contains the 60-62 codons of the human \textit{n-ras} proto-oncogene. Point mutations at the 61 codon (CAA), which have been linked to UV-irradiation, can lead to oncogene activation.\textsuperscript{16}
4.2. Results

4.2.1. Steady-state Circular Dichroism and Absorption Spectra

Figure 4.1 shows the structure of the ras61 oligonucleotide determined by a combined $^1$HNMR and constrained molecular dynamics simulation study. CD spectra of strand A and strand B are shown in Figure 4.2. Also shown is the CD spectrum for a solution containing an equimolar mixture of strand A and strand B. This spectrum exhibits characteristic B-form DNA bands and confirms that the A·B duplex is successfully formed in our experimental conditions. Figure 4.3 shows melting temperature curves from 20 - 80°C. Strand A and strand B have broad melting curves, supporting the conclusion that these non-self-complementary sequences are single-stranded in solution. The duplex solution exhibit a sigmoidal melting curve characteristic of a helix-to-coil transition in solution and has a melting temperature of approximately 47°C.

UV/Vis absorption spectra taken before transient absorption measurements, after 5 scans, and after 10 scans are shown in Figure 4.4. The absorption spectrum for strand B shows a significant decrease in amplitude at the maximum after multiple scans. Figure 4.5 compares the difference absorption spectrum recorded before and after UV irradiation for strand B with the difference absorption spectrum for a sample of TpT where the cis-syn thymine dimer is the major product. The similar spectra suggest that changes in absorption spectrum of strand B are mainly due to formation of pyrimidine dimer following irradiation with 266 nm light. As a result of this degradation, only the first 5 scans were averaged for the transient signals shown in Figures 4.6 and 4.7. Each transient
scan from scan 1 to 10 was compared for any effect the photodegradation may have on the signals. Other than a decrease in amplitude, which is expected given the decrease in UV absorbance at the pump wavelength, no difference in the kinetics is observed. Less photodegradation is observed when in the A·B duplex.

4.2.2. Transient Absorption Signals

Transient absorption signals for strand A, strand B, and the A·B duplex excited at 266 nm and probed at 570 and 250 nm are shown in Figure 4.6 and 4.7, respectively. The probe wavelength of 570 nm monitors excited-state relaxation and the wavelength of 250 nm follows repopulation of the ground state. Near mirror symmetry is observed between the signals at 570 nm (Figure 4.6) and 250 nm (Figure 4.7). Fitting these signals proved a challenge: fit to 3 and 4 exponentials had similar $\chi^2$ values and residuals traces, but gave significantly different time constants. In order to avoid overfitting the data, a mathematical strategy was used to select the appropriate fitting model, as discussed in Section 2.6.\cite{19} Multiple fitting models were used and the one that gave the minimum Akaike information criterion (AICc) value was reported. Using this strategy, the signals for strand A and the A·B duplex were fit to 4 exponentials and an offset. Fit parameters are listed in Table 4.1. All the signals were fit to two fast components with lifetimes of $0.43 \pm 0.15$ and $3.4 \pm 0.3$ ps. The fastest component ($0.43 \pm 0.15$ ps) has a positive amplitude. The strand A signal also has two long-lived decay components of $64 \pm 30$ and $300 \pm 100$ ps. The A·B duplex signal also has two long-lived decay components, with lifetimes of $27 \pm 6$, and $440 \pm 260$ ps. The strand B signal only has one slow decay
component with a lifetime of $27 \pm 6$ ps. A small offset of 6-10\% is observed at long time delays in all the bleach signals.

Another method to obtain lifetime information from multi-exponentials signals is to determine the time required for the signal to decay a certain percent from its initial time zero value.$^{20}$ The $\tau_{75}$ values, where the signal amplitude is 75\% of the original amplitude, are 114, 26, and 19 ps for the strand A, strand B, and A·B duplex signals, respectively.

4.3. Discussion

4.3.1. Sequence Disorder in the ras61 Oligonucleotide

The excited-state dynamics of many different DNA oligomers have been studied-ranging from homo-adenine polymers to GC-containing oligomers (Chapter 3) - but the degree of sequence disorder has been limited.$^{21}$ Even in the most recent work by Schwalb and Temps,$^{13}$ where one of the focuses of the study was the effect of sequence on excited-state dynamics, the sequences studied contained only one or two types of bases per strand and up to three distinct doublets. A doublet is a pair of adjacent bases read in the 5'→3' direction. There are 16 possible unique doublets for the four natural DNA bases in single-stranded DNA. In this study, strand A contains 7 of these possible doublets (AA, AG, AC, GA, GG, CA, CG) and strand B contains the 7 complementary doublets (TT, TC, TG, CT, CC, GT, GC). In the A·B duplex, 14 of the 16 possible doublets are present, with only AT and TA missing.
4.3.2. Transient Absorption of DNA with Significant Sequence Disorder

Long-lived excited-states are observed in strand A, strand B, and the A-B duplex. Different relaxation times are observed for the individual single strands, with $\tau_{75}$ values of 114 and 26 ps for strand A and strand B, respectively. Strand A, which is rich in A and G bases, has a slower relaxation to the ground state than the pyrimidine-rich strand B. The presence of a long-lived decay component in strand B is interesting because long-lived states have not been reported for pyrimidine-rich single strands.\(^3,13\) The $27 \pm 6$ ps lifetime is similar to that of the $^{1}n\pi^{*}$ state in cytosine;\(^22\) however, this state is ruled out as the source of this long-lived signal since this component is also present at 570 nm (Figure 4.6) where the $^{1}n\pi^{*}$ state of cytosine does not absorb.\(^22\)

The different decay lifetimes reported for the individual genomic strands highlight the importance of base sequence on the excited-state dynamics. Although the transient signals were fit to 3 and 4 exponentials, the resulting time constants likely represent an average of similar decays for different exciplex states within each strand. Strand A contains a majority of AA- and AG-containing doublets, while strand B is composed of CC, CT and TT doublets. The longer lifetimes reported for strand A is consistent with the longer lifetimes expected for the AA and AG doublets. Lifetime of 105 ps are reported for both AA and AG dinucleosides.\(^4\) In contrast, the CC excimer state has a lifetime of 10 ps, while no long-lived excited states have been observed in single-stranded (dT)\(^{18}\).\(^3,20\) The lifetime of the CT exciplex state has yet to be reported. A more complete set of exciplex lifetimes and a better understanding of how neighboring bases affect excited-
state decay are needed to better understand the lifetimes of the different decay components in this disordered systems.

It has been shown that exciplex lifetime is correlated with the energy of the charge transfer state, with longer lifetimes proposed for higher-energy exciplex states.\(^4\) The presence of long-lived signals in these systems indicates that a significant number of higher-energy exciplex states are populated. This suggests that energy transfer from high-energy exciplex states to lower-lying exciplex states is not a dominant process in strand A and the A·B duplex. However, the significant amount of ultrafast relaxation seen in the A·B duplex bleach signals could be explained by electron transfer or funneling to lower energy states.

The larger offset seen in the transient signals for strand B compared to strand A and the A·B duplex likely results from the increased photochemistry in this strand (Figure 4.4). Strand B consists of a large number of sites (CC, CT, TC, and TT) that are susceptible to pyrimidine dimer formation. The formation of photoproducts at these sites would prevent the excite-state population from fully returning to the ground state and result in an offset of the signal at 250 nm. A concern with dimer formation in the strand is that it could alter the structure and affect dynamics; however, no difference in kinetics was observed between scans. Longer-lived excited states with ns lifetimes have also been recently reported in GC- containing\(^{11,12}\) and genomic DNA by Vayá \textit{et al.}\(^{11}\) The nature of these longer-lived excited states is unclear, but population of such states would slow population recovery of back to the ground state and also result in an offset in the ground-state bleach signal at 250 nm.
4.3.3. Ultrafast Decay in a Well-stacked System

At early times, the transient bleach signals in Figure 4.7 are seen to grow in with a time constant of $0.43 \pm 0.15$ ps. Similar behavior was observed in 9-methyladenine in H$_2$O at 250 nm and assigned to absorption by a high energy absorption band that is formed following ultrafast relaxation to the ground state.$^{23}$ This component, along with the large amplitude for the $3.4 \pm 0.3$ ps component seen in the bleach signals (Figure 4.7), suggests that there is a significant amount of ultrafast relaxation to the ground state in all three systems. Similar signals in homo-adenine polymers$^3$ and dinucleotides$^4$ have been assigned to monomer-like decay from unstacked bases. The relative amplitudes of the 3.4 ps component are 46 $\pm$ 10, 47 $\pm$ 14, and 51 $\pm$ 9% for strand A, strand B, and the A·B duplex (Table 4.1).

The large amplitude of the $3.4 \pm 0.3$ ps component in the A·B duplex signal is surprising given that this component has been assigned to decay in unstacked bases.$^3$ Using a nearest-neighbor thermodynamic model developed by SantaLucia, approximately 93% of the bases in this duplex are predicted to be stacked at room temperature.$^{24}$ The terminal bases pairs of the A·B duplex are GC base pairs, which are more stable and open less than AT base pairs.$^{25}$ Approximately 10% of the terminal GC base pairs are estimated to be open in solution at 25°C.$^{26}$ Assuming almost full stacking in the interior bases and 10% fraying at the terminal bases, only about 2% of all the bases in the duplex are unstacked. Another possible source of unstacked bases is excess single-strands in solution. In the ideal scenario, every strand A will be base paired with every strand B in solution, but in the actual experiment, there will be excess of one strand in solution that
can also be a source for unstacked bases. Even if there were 10% excess of one strand in solution, that would only account for up to 5% of the bases being unstacked, assuming the extreme case where no stacking is present in the excess strands. The amount of unstacked bases expected in the A-B duplex is not consistent with approximately half the ground-state population recovering on the ultrafast time scale. The large amplitude of the fast component suggests that ultrafast decay pathways are still present in well-stacked DNA. Recent computational studies have suggested that subpicosecond monomer-like channels are still present in stacked bases.\textsuperscript{27} Improta \textit{et al.} proposed that excimer formation may not be the dominant pathway in all stacked systems.\textsuperscript{27} For TT stacked bases relaxation to the ground state is proposed to occur by monomer-like internal conversion through conical intersections rather than form excimer states.\textsuperscript{27}

\textit{4.3.4. Excited-state Yields}

The transient absorption signals shown in Figure 4.7 were measured in back-to-back experiments in equal absorbance solutions under the same experimental conditions. The same number of initial excited states are expected in each sample, so it is of interest to compare signal amplitudes of the individual strands and the duplex. A nearest-neighbor model has been developed for DNA systems comprised of multiple base chromophores to predict extinction coefficients.\textsuperscript{28-31} The extinction coefficient of single strands is traditionally calculated by summing the extinction coefficients of base doublets and subtracting the extinction coefficients of the individual bases to correct for overcounting. Using the simplified form given in Tataurov \textit{et al.}
the extinction coefficients for strand A and strand B were calculated to be 118300 and 90800 M$^{-1}$ cm$^{-1}$, respectively, at the excitation wavelength of 267 nm. These values do not take into account changes to the extinction coefficients that occur upon base pairing. A crude model based on the extinction coefficient for each strand would predict 0.519 probability of strand A excitation ($99321/(99322 + 91996)$ M$^{-1}$ cm$^{-1}$) and a 0.481 probability of exciting strand B in the duplex. Although it is by no means certain whether excitations are restricted to one strand or the other and the involvement of excitonic states complicates matters, the weighted signal average of strand A and strand B roughly reproduces the A$\cdot$B duplex signal (Figure 4.8). The difference between the average trace and the A$\cdot$B duplex signal is shown in the upper panel of Figure 4.8.

The overall agreement suggests that interstrand base pairing does not contribute significantly to the excited-state dynamics in duplexes, as has been reported previously in non-alternating AT-DNAs.$^3$ Also the lifetimes of native DNA has been proposed to be determined mainly by the lifetime of pyrimidine bases,$^{32}$ which is shown not to be the case. However, there are interesting differences between the A$\cdot$B duplex signal and the average signal. The A$\cdot$B duplex has a greater amplitude at early times compared to the average signal and a lower amplitude in the 10 - 100 ps range compared to the average signal. The fast 3.4 ps component has a similar contribution in the duplex as in the single strands (Table 4.1) and that overall contribution from the long-lived states is not greater in the duplex. This is surprising since a greater degree of stacking is expected for the
duplex than in strand A or pyrimidine-rich strand B that is only weakly stacked (Figure 4.3). The absence of the 64 ps state observed in strand A, but not in the A-B duplex suggests that this state may be quenched in the duplex.

4.4. Conclusions

Long-lived excited states are observed in single- and double-stranded DNA oligonucleotides that have significant base sequence disorder. The observation of these long-lived states indicates that these states are not an artifact of well-ordered model DNA systems. The presence of long-lived states in genomic DNA highlights the importance of studying the photochemical implications of these states. In the A-B duplex, base pairing has a relatively small effect on the overall excited-state dynamics and a large fraction of all decay events occurs by ultrafast monomer-pathways. This demonstrates that ultrafast deactivation pathways are still of importance in well-stacked DNA systems.

References


(4) Takaya, T.; Su, C.; de La Harpe, K.; Crespo-Hernández, C. E.; Kohler, B. 


Figure 4.1. Solution structure of ras6l oligodeoxynucleotide refined from $^1$HNMR data (PDB: 1AGH).
Figure 4.2. CD spectra of single-stranded and duplex genomic DNA in 25mM phosphate buffer with 100 mM NaCl at 20°C.
Figure 4.3. Melting temperature curves for strand A (triangle), strand B (squares), and the A·B duplex (circles) from 20 to 80 °C.
Figure 4.4. Absorption spectra recorded before transient absorption measurements (red), after 5 scans (green), and after 10 scans (blue).
Figure 4.5. Difference absorption spectra recorded before and after UV irradiation for TpT (blue) and for strand B (green).
Figure 4.6. Transient absorption signals (266 nm / 570 nm) for single-strand and duplex genomic DNA. Transients were corrected for solvated electron absorption. Best-fit curves are shown by solid lines.
Figure 4.7. Back-to-back transient absorption signals (266 nm pump / 250 nm probe) for single-strand and duplex genomic DNA. Best-fit curves are shown by solid lines.
Figure 4.8. Top panel: Difference of the A-B duplex signal and the weighted average for the single-strands A and B. Bottom panel: Transient signals from the single-strand and duplex genomic DNA compared to the weighted average of the single-strand signals.
Table 4.1. Lifetimes and amplitudes (in parentheses) from globally fitting the transient bleach signals shown in Figure 4.7. Boldface values in the same column were linked during global fitting. All uncertainties are twice the standard error.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\tau_0$ (ps)</th>
<th>$\tau_1$ (ps)</th>
<th>$\tau_2$ (ps)</th>
<th>$\tau_3$ (ps)</th>
<th>$A_\infty$</th>
</tr>
</thead>
</table>
| Strand A    | \textbf{0.43 \pm 0.15}  
(0.62 \pm 0.14) | \textbf{3.4 \pm 0.3}  
(-1.47 \pm 0.08) | 64 \pm 30  
(-0.45 \pm 0.20) | 300 \pm 100  
(-0.51 \pm 0.21) | -0.13 \pm 0.02 |
| Strand B    | \textbf{0.43 \pm 0.15}  
(0.43 \pm 0.11) | \textbf{3.4 \pm 0.3}  
(-0.64 \pm 0.08) | \textbf{27 \pm 6}  
(-0.28 \pm 0.04) | -- |
| A-B Duplex  | \textbf{0.43 \pm 0.15}  
(0.59 \pm 0.12) | \textbf{3.4 \pm 0.3}  
(-1.32 \pm 0.10) | \textbf{27 \pm 6}  
(-0.49 \pm 0.07) | 440 \pm 260  
(-0.16 \pm 0.03) | -0.09 \pm 0.02 |
Chapter 5: Vibrational Studies of AT-containing Oligonucleotides

5.1. Introduction

Base stacking and base pairing interactions are important in the structure of duplex DNA. The role that base stacking and base pairing play in the dissipation of excess electronic energy in DNA has been of interest for many years. Base-stacking interactions in single- and double-stranded DNA oligonucleotides result in long-lived excited electronic states not observed in monomeric nucleobases.\(^1\) These states are proposed to be exciplex states with charge-transfer (CT) character formed between a pair of \(\pi\)-stacked bases.\(^2\) The importance of base stacking to long-lived excited-states has been established in single- and double-stranded DNA, but the effect of base pairing on DNA excited-state dynamics is still unclear.

Deuterium isotope effects have been observed in the excited-state dynamics of alternating AT and GC oligomers. In \((\text{dAdT})_9'(\text{dAdT})_9\), the long-lived excited state decays with a lifetime of \(66 \pm 14\) ps in \(\text{H}_2\text{O}\) and \(150 \pm 30\) ps in \(\text{D}_2\text{O}\).\(^3\) In \((\text{dGdC})_9'(\text{dGdC})_9\), the excited-state decay is well fit with a single exponential with a time constant of \(6.3 \pm 0.4\) ps in \(\text{H}_2\text{O}\) and with two exponentials with time constants of \(6.3 \pm 0.4\) and \(22 \pm 4\) ps in \(\text{D}_2\text{O}\) (as discussed in Chapter 3).\(^4\) Blocking base pairing through base modification eliminates the isotope effect in a single-stranded alternating GC system,
supporting the hypothesis that an interstrand process contributes to the excited state
dynamics in (dGdC)$_9$-(dGdC)$_9$.

However, no isotope effect was also observed for non-alternating sequences with the same Watson- Crick (WC) base pairing arrangements. These observations were reconciled with a proposed model where proton transfer (PT) is initiated by the formation of an intrastrand exciplex state with significant charge transfer, such as in the case of the alternating sequences but not the non-alternating one.

Although these studies suggest that interstrand process affect the excited-state dynamics in duplex DNA, there is no direct evidence for proton transfer.

A powerful tool for investigating proton transfer in DNA is time-resolved infrared spectroscopy. Using a mid-IR probe, vibrational absorption spectra of a sample can be monitored in time following UV excitation. Vibrational spectra typically have narrower features and are more sensitive to molecular structure than electronic spectra. These properties make UV-pump/mid IR-probe spectroscopy an ideal method for identifying unique bands that can be used to characterize transient excited states. The carbonyl and double-bond stretching region from 1550 - 1800 cm$^{-1}$ of a series of A- and T-containing oligonucleotides were studied following UV excitation using time-resolved infrared spectroscopy. Vibrational bands in this region have been shown to be sensitive to base pairing interactions. Possible marker bands for the AA excimer state in single-stranded (dA)$_{18}$ are observed at 1545 and 1617 cm$^{-1}$. Strong positive bands observed at early times in mid-IR transient spectra of (dAdT)$_9$-(dAdT)$_9$, but not in other AT containing systems, are proposed to arise from PT within AT base pairs.
5.2. Results

5.2.1. Mid-IR Transient Absorption Spectra of Single-stranded (dA)\textsubscript{18} and (dT)\textsubscript{18}

TRIR spectra of (dA)\textsubscript{18} in phosphate D\textsubscript{2}O buffer obtained at time delays from 1 ps to 3 ns following 270 nm excitation are shown in Figure 5.1. Transient bleach bands are observed at two wavenumbers: a strong band at 1629 cm\textsuperscript{-1} and a weak band at 1578 cm\textsuperscript{-1}. Using the AICc fitting strategy discussed in Section 2.5.2, the bleach kinetics at 1629 cm\textsuperscript{-1} were well fit to 3 exponentials with lifetimes of 5.8 ± 0.3 ps, 147 ± 26 ps, and 1.2 ± 0.2 ns. The weak bleach at 1571 cm\textsuperscript{-1} has a single exponential decay with a lifetime of 339 ± 129 ps. Positive transient bands are present at lower wavenumbers, with a positive band at the lower wavenumbers edge of the bleach band at 1629 cm\textsuperscript{-1} and a broad flat band around 1545 cm\textsuperscript{-1}. The band at 1550 cm\textsuperscript{-1} is present until around 200 ps: it has a biexponential decay with time constants of 3.5 ± 1.3 and 87 ± 60 ps. The positive band at 1629 cm\textsuperscript{-1} narrows and shifts to higher wavenumber as a function of time, shown in the difference spectra in Figure 5.2. Kinetic traces at 1550, 1578, and 1629 cm\textsuperscript{-1} are given in Figure 5.3. The lifetimes and relative amplitudes for fits of the transient signals are given in Table 5.1. Although this positive band appears to decay to the baseline by 30 ps in Figure 5.1, Lorentzian fits of the spectra reveal that the positive band has significant amplitude until 1 ns after excitation. Transient spectra at time delays of 5 ps, 30 ps, and 500 ps were fit to a sum of Lorentzians, shown in Figure 5.4. A negative band centered at 1629 cm\textsuperscript{-1} and a positive band at 1619 cm\textsuperscript{-1} that narrows with time are present at all three time delays. An additional weak bleach band at 1595 cm\textsuperscript{-1} is also present after 30 ps.
The TRIR spectra of (dT)$_{18}$ in phosphate D$_2$O buffer obtained at time delays from 1 ps to 3 ns following 270 nm excitation is shown in Figure 5.5. Three bleach bands at 1630, 1663 and 1696 cm$^{-1}$ dominate the transient spectra. The bleach signals at 1630, 1663 and 1696 cm$^{-1}$ are shown in Figure 5.6 and the fit parameters are given in Table 5.1. The bands at 1663 and 1696 cm$^{-1}$ recover to the ground state with essentially identical dynamics: the bleaches are well fit to 3 exponentials with time constants of 2 ps, 32 ± 13 ps, and 0.9 ± 0.3 ns. In contrast, the band at 1630 cm$^{-1}$ has a faster initial recovery with time constants of 0.5 ± 0.6 ps, 7.7 ± 2 ps, and 0.9 ± 0.3 ns. At long times, the signal does not return fully to baseline. Figure 5.7 shows transient spectra at time delays of 5 ps, and 30 ps fit to a sum of Lorentzians. The spectra at 5 and 30 ps contain 3 prominent bands. The bands at 1664 and 1631 cm$^{-1}$ have negative amplitude and the maxima remains constant with time, the positive band at 1651 cm$^{-1}$ has almost equal and opposite amplitude at 1631 cm$^{-1}$ and shifts to higher wavenumbers with time.

The difference spectra in Figure 5.8 shows transient bands at lower wavenumbers of 1611 and 1644 cm$^{-1}$ at early times, these bands have the same spacing (30 cm$^{-1}$) as the steady state bands at 1632 and 1663 cm$^{-1}$. This suggests that after excitation the bands at 1632 and 1663 cm$^{-1}$ undergo a 20 cm$^{-1}$ shift to lower wavenumbers at early times. These transient bands shift back to higher wavenumbers by approximately 15 ps. The band at 1696 cm$^{-1}$ does not undergo as extreme of a shift in band position, with a transient feature appearing at 1688 cm$^{-1}$.  

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5.2.2. Mid-IR Transient Absorption Spectra of (dA)_{18} \cdot (dT)_{18} and (dAdT)_9 \cdot (dAdT)_9 at Elevated Sample Temperatures

DNA samples for the UV-pump/mid IR-probe experiments were held in a custom-built spinning sample cell with a 100 µm spacer in order to minimize solvent absorption, as discussed in Section 2.3. Significant changes to the transient absorption spectra of the duplexes (dA)_{18}(dT)_{18} and (dAdT)_9(dAdT)_9 were observed following prolonged exposure to pump pulses (Figure 2.6). These spectral changes were attributed to melting of the duplexes resulting from sample heating by the pump pulses. At these elevated sample temperatures, the spectrum of (dA)_{18}(dT)_{18} at 1 ps after excitation resembles the steady-state FTIR spectrum of (dA)_{12}(dT)_{12} at temperatures of 50°C and greater.\footnote{This suggests that we have an increase in temperature of 20 - 30 °C following prolonged exposure to the pump pulses. Limiting the exposure of the sample to the pump pulses and translating the cell to a new sample position for each delay time successfully minimized sample heating and duplex melting. Although sample heating makes collecting room temperature duplex data more challenging, it also allows us to study duplex systems at elevated temperatures where base pairing interactions are disrupted.}

The TRIR spectra of (dA)_{18}(dT)_{18} and (dAdT)_9(dAdT)_9 obtained at elevated sample temperatures are shown in Figure 5.9. Similar spectra are observed for both (dA)_{18}(dT)_{18} and (dAdT)_9(dAdT)_9. In (dA)_{18}(dT)_{18}, the transient spectra at early time contains three main bleach bands at 1624, 1666 and 1696 cm^{-1}. In (dAdT)_9(dAdT)_9, the
three main bleach bands are at 1620, 1666 and 1696 cm$^{-1}$. Transient bleach signals at these specific wavenumbers are shown in Figure 5.10 with fit parameters given in Table 5.2. For (dA)$_{18}$·(dT)$_{18}$, all three bleach signals were globally fit to 3 exponentials with lifetimes of $2.5 \pm 0.6$ ps, $28 \pm 18$ ps and $1.2 \pm 0.4$ ns, respectively. Similar lifetimes are observed for single-stranded (dT)$_{18}$ and the bleach at 1620 cm$^{-1}$ in (dAdT)$_9$·(dAdT)$_9$. For (dAdT)$_9$·(dAdT)$_9$, the bleaches are well fit to a biexponential decay with an offset. The bleach at 1620 cm$^{-1}$ recovers with lifetimes of $2.9 \pm 0.3$ and $44 \pm 13$ ps. The bleaches at 1666 and 1697 cm$^{-1}$ have similar kinetics and recover with lifetimes of $2.9 \pm 0.3$ and $102 \pm 23$ ps, respectively. The $102 \pm 23$ ps is similar to the $150 \pm 30$ ps lifetime reported for UV/UV transient absorption studies of (dAdT)$_9$·(dAdT)$_9$ in D$_2$O; although, the relative amplitude of this component is only about half of the value reported for the UV-pump / UV-probe transient absorption study (Table 5.2).$^3$

The transient spectra at 1 ps in Figure 5.9 differ significantly from the FTIR spectra of both (dA)$_{18}$·(dT)$_{18}$ and (dAdT)$_9$·(dAdT)$_9$. The FTIR spectrum of (dA)$_{18}$·(dT)$_{18}$ contains four vibrational bands at 1624, 1640, 1662 and 1696 cm$^{-1}$. The transient spectrum, with its three prominent bands, more closely resembles the sum of the individual single-strands (dT)$_{18}$ and (dA)$_{18}$ (see Figure 5.1 and 5.1), having a broader bleach band around 1624 cm$^{-1}$, and lacking the band at 1640 cm$^{-1}$ and the increased amplitude of the band at 1696 cm$^{-1}$. A rough fit of the spectrum at a time delay of 5 ps time to a sum of Lorentzians is shown in Figure 5.11. The addition of a large positive offset significantly improves the fit of the bleach band at 1624 cm$^{-1}$; however, a physical reason for such an offset is unlikely and more likely indicates that addition positive bands
contribute to this complex spectrum. Without this offset, at least four Lorentzians are needed to fit the transient spectrum. Notably, the bleach band around 1624 cm$^{-1}$ can be roughly fit with two Lorentzians centered 1621 and 1630 cm$^{-1}$.

5.2.3. Mid-IR Transient Absorption Spectra of (dA)$_{18}$ ⋅ (dT)$_{18}$ at Room Temperature

The transient absorption spectra of the duplex (dA)$_{18}$⋅(dT)$_{18}$ at room temperature were measured under conditions that limited the exposure of the sample to the pump pulses, as described in Section 2.3. A limited number of time delays were collected, a new position in the sample was probed for each delay time, and the sample was only exposed to the pump pulses during data acquisition in order to minimize sample heating. The similar transient spectra obtained for duplex (dAdT)$_9$⋅(dAdT)$_9$ under these conditions, shown in Figure 5.14, and for poly(dAdT) recorded using a flow cell confirm that minimal duplex melting is occurring.

Figure 5.12 shows the TRIR spectra of duplex (dA)$_{18}$⋅(dT)$_{18}$ at room temperature in 100 mM phosphate D$_2$O with 0.25 M NaCl at select time delays from 1 to 3000 ps following 270 nm excitation. Transient bleach bands are observed at 1624, 1641, 1663, and 1700 cm$^{-1}$. The frequencies of these bleaches are close to those in the ground state IR spectrum; although, the relative amplitudes of the bleaches do not match. The signals at the bleach maxima are shown in Figure 5.13 and the fit parameters are given in Table 5.3. All four bands decay with a fast component, a slower common 18 ± 8 ps component and an offset. An offset is observed over the entire spectrum. The bleach maxima and bandwidths remain nearly constant with time for these bands. A broad positive feature appears from 1610 to 1580 cm$^{-1}$ and decays to the ground state by 100 ps. At early times,
positive features are also observed at 1648 and 1677 cm\(^{-1}\), indicating the presence of strong underlying positive bands.

5.2.4. Mid-IR transient absorption spectra of (dAdT)\(_9\) \(\cdot\) (dAdT)\(_9\) at Room Temperature.

The TRIR spectra of duplex (dAdT)\(_9\) \(\cdot\) (dAdT)\(_9\) at room temperature in 100 mM phosphate D\(_2\)O with 0.25 M NaCl at select time delays from 1 to 1100 ps following 270 nm excitation are shown in Figure 5.14. Two bleach bands are present at 1620 and 1669 cm\(^{-1}\) with an additional bleach band appearing at the edge of the spectral window around 1690 cm\(^{-1}\). The bleach at 1620 cm\(^{-1}\) is well fit to two exponentials and an offset with time constants of 4.2 ± 0.3 and 217 ± 33 ps. The maximum of this band does not change as it decays. The bleach at 1669 cm\(^{-1}\) decays with similar time constants, but requires an additional component. The 1669 cm\(^{-1}\) bleach recovers with time constants of 3.5 ± 0.7, 20 ± 9, 182 ± 16 ps and an offset. The position of these bleach bands are similar in to those in the ground-state IR spectrum of (dAdT)\(_9\) \(\cdot\) (dAdT)\(_9\), also shown in Figure 5.14. However, a striking feature of these spectra is the two positive bands in the middle of the spectral window at 1633 and 1647 cm\(^{-1}\). In order to exceed the strong ground state bleaching expected in this region, these transient bands must be intense. These bands exhibit similar kinetics after 3 ps, recovering with time constants of 3.7 ± 1.4 and 76 ± 15 ps. A broad positive feature is also observed from 1585 to 1610 cm\(^{-1}\); this band recovers to the ground state within 500 ps. The signal at 1586 cm\(^{-1}\) is well fit to a biexponential with lifetimes of 1.9 ± 0.4, 83 ± 9 and a small offset. The transient signals for (dAdT)\(_9\) \(\cdot\) (dAdT)\(_9\) are shown in Figure 5.15 and the fit parameters are given in Table 5.3.
5.3. Discussion

5.3.1. Marker Band for Excimer State in (dA)$_{18}$

Long-lived excited states have been observed in A-containing homopolymers using UV/UV and UV/Vis transient absorption spectroscopy. Biexponential decays reported for these systems are assigned to a fast monomer-like component that arises from unstacked bases and a long-lived 100 - 150 ps component assigned to excimer states formed between stacked bases. The bleach band at 1629 cm$^{-1}$ in Figure 5.1, assigned to bleaching of the ground state IR band in (dA)$_{18}$, exhibits a similar biexponential decay. The observed 5.4 ± 0.4 ps component is slower than the 2.9 ps vibrational cooling time reported for UV/UV transient absorption studies in D$_2$O; however, it is similar to the longer recovery time of 4.3 ± 0.2 ps reported for UV/mid-IR studies of dAMP and poly(A) in D$_2$O. The 148 ± 38 ps matches well with the lifetimes reported for the AA excimer state. These results are similar to the UV/mid IR study of poly(A) in D$_2$O. An additional 1.2 ns component is observed that was not previously reported.

In addition to the strong bleach band at 1629 cm$^{-1}$, two positive transient features of interest appear at 1617 and 1545 cm$^{-1}$. The strong positive band at 1617 cm$^{-1}$ is observed at lower frequencies to the ground-state bleach in Figure 5.1. It appears to decay to the ground state by 8 ps; however, fitting the transient spectra of (dA)$_{18}$ to a sum of Lorentzians reveals that this transient band is present even after 500 ps, as shown in Figure 5.4. This band narrows but remains centered at 1617 cm$^{-1}$ from 5 ps to 500 ps; it does not shift to higher wavenumbers as would be expected for intramolecular vibrational redistribution processes. A similar offset in mid-IR spectra of poly(A) at long times was
assigned to an underlying transient band from an excimer state between 1615 and 1630 cm$^{-1}$. A broad positive band is also present at frequencies lower than 1570 cm$^{-1}$. This band has a biexponential decay with time constants of $3.5 \pm 1.3$ and $87 \pm 60$ ps. The 3.5 ps component is likely due to the decay of an underlying hot ground state band, while the 87 ps component is similar to the lifetime associated with the AA excimer state. The presence of this band at long times suggests that it may also be an excimer band.

5.3.2. Transient Absorption Spectra of AT-DNA at Elevated Sample Temperatures

Sample heating in the UV-pump / mid IR-probe experiments is proposed to result in melting of the (dA)$_{18}$·(dT)$_{18}$ and (dAdT)$_9$·(dAdT)$_9$ duplexes. The mid-IR transient spectra of (dA)$_{18}$·(dT)$_{18}$ and (dAdT)$_9$·(dAdT)$_9$ at elevated sample temperatures are strikingly similar between 1550 and 1750 cm$^{-1}$ spectra despite significant differences in ground-state spectra (Figure 5.9). The FTIR spectrum of (dA)$_{18}$·(dT)$_{18}$ consists of four vibrational bands at 1622, 1640, 1663 and 1695 cm$^{-1}$ in the spectral region of 1500 – 1700 cm$^{-1}$. The 1642 cm$^{-1}$ band, which is not present in the individual single strands, arises from a blue shift in the thymine band at 1632 cm$^{-1}$ upon WC base pairing between the adenine and thymine bases. The FTIR spectrum of (dAdT)$_9$·(dAdT)$_9$ (Figure 5.9) has three vibrational bands at 1622, 1641, 1666, and 1690 cm$^{-1}$. The main difference in FTIR spectra between (dA)$_{18}$·(dT)$_{18}$ and (dAdT)$_9$·(dAdT)$_9$ is the relative amplitude of the bands: in (dA)$_{18}$·(dT)$_{18}$ they have roughly the same amplitude, while in (dAdT)$_9$·(dAdT)$_9$ the band at 1666 cm$^{-1}$ dominates the spectrum. The transient spectra for both systems have similar features with only three bleach bands at 1624, 1666 and 1696 cm$^{-1}$ shown in
Figure 5.9. The noticeable absence of the 1642 cm$^{-1}$ band in the transient spectra suggests that we are observing a disruption of WC base pairing interactions.

Band positions and intensities are highly sensitive to base pairing interactions in this IR region, with smaller spectral changes associated with base stacking.$^{5,11,12}$ It is possible that the differences observed between the FTIR and the transient absorption spectra may result from the formation of higher-ordered structures with different base pairing motifs. Non-alternating A- and T-tracts can form a triple helix with an additional strand base paired in a Hoogsteen or reverse Hoogsteen scheme.$^{13}$ The possibility that such structures are being formed at the higher concentrations needed for these experiments was eliminated by comparing the transient spectra with reported FTIR spectra of different base pairing arrangements.$^{12}$ The similar transient spectra observed in Figure 5.9 are not the result of non-WC base pairing arrangements.

Rather, the differences observed between the FTIR spectra and the transient spectra are similar to those seen at elevated temperatures. In steady-state experiments by Liquier et al., increasing temperatures from 5-70°C resulted in 3 distinct changes in the FTIR spectra of (dA)$_{18}$·(dT)$_{18}$: 1) the disappearance of the band at 1641 cm$^{-1}$, 2) an increase in the adenine band at 1622 cm$^{-1}$, and 3) a decrease in amplitude and broadening of the band at 1696 cm$^{-1}$. As shown in Figure 2.6 and discussed in Section 2.3, changes in the transient spectrum of (dA)$_{18}$·(dT)$_{18}$ at a time delay of 1 ps at different exposure times to the pump pulses matches those observed for melting of DNA duplexes. The transient spectra at 1 ps in Figure 5.9 for (dA)$_{18}$·(dT)$_{18}$ and (dAdT)$_{9}$·(dAdT)$_{9}$ at elevated temperatures are similar to FTIR spectra of similar systems at temperatures of 50 °C and
greater. The spectra at these temperatures are assigned to the single strands. DNA melting studies have shown that the unpairing of bases occurs over a narrow temperature range while the unstacking of bases occurs over a broad range, with base pairing completely disrupted before base stacking. At these elevated sample conditions, dA_{18}·dT_{18} and (dAdT)_{9}·(dAdT)_{9} are single-stranded in solution.

The transient signals for dA_{18}·dT_{18} and (dAdT)_{9}·(dAdT)_{9} recorded at elevated temperature in Figure 5.10 can provide insight into the degree of structural disruption and melting in the system. The vibrational bands are not as sensitive to stacking; yet the kinetics of these bands should provide information of the degree of stacking present at these elevated sample temperatures. In the (dAdT)_{9}·(dAdT)_{9} sample at elevate sample temperatures, the bleaches at 1666 and 1697 cm\(^{-1}\) have similar kinetics and recover with lifetimes of 2.9 ± 0.3 and 102 ± 23 ps. The 102 ± 23 ps is similar to the 150 ± 30 ps lifetime reported for UV/UV transient absorption studies of duplex (dAdT)_{9}·(dAdT)_{9} in D\(_2\)O; although, the relative amplitude of this component is only about half of the value reported for the UV/UV study. The presence of long-lived states in this melted duplex sample indicates that at an elevated temperature we have disrupted base pairing interactions while maintaining significant π-stacking interactions. In (dA)_{18}·(dT)_{18}, the slow lifetime components present in dTMP (547 ps) and (dT)_{18} (32 ps) make it difficult to determine whether significant base stacking is present in this system.

5.3.3. Marker Bands for Proton Transfer in (dAdT)_{9}·(dAdT)_{9}

The mid-IR transient spectra of (dA)_{18}, (dT)_{18}, (dA)_{18}·(dT)_{18}, and (dAdT)_{9}·(dAdT)_{9} at room temperature were compared to determine whether there were
any transient bands that could be assigned to AT-exciplex states or PT in (dAdT)_9·(dAdT)_9 in the 1550 and 1750 cm⁻¹ spectra window. In Figure 5.14, two positive bands at 1630 and 1640 cm⁻¹ are observed in the spectra of (dAdT)_9·(dAdT)_9, but not in the other systems studied (Figures 5.1, 5.5, and 5.12). These bands are also not present in mid-IR transient absorption spectra of the individual adenine and thymine bases.⁶,⁹ The absence of these transient bands in the spectra of these systems indicates that they arise from excited states unique to (dAdT)_9·(dAdT)_9. These bands match those seen in mid-IR transient spectra of poly(dAdT).⁶

The oligomer (dAdT)_9·(dAdT)_9 differs from the other AT-containing systems studied in that it has both 1) repeating 5'-AT-3' and 5'-TA-3' stacks and 2) AT WC base pairs. UV-pump/UV-probe experiments have shown that long-lived states assigned to intrastrand CT states are present in significant yields in (dAdT)_9·(dAdT)_9.³,¹⁶ A kinetic isotope effect was also observed in the alternating, but not the non-alternating, AT-DNA. It was proposed that AT-exciplex state with significant charge-transfer character initiates interstrand proton transfer.³ The positive bands in the mid-IR transient spectra of (dAdT)_9·(dAdT)_9 could be possible marker bands for the AT-exciplex state or proton transfer.

To determine whether the bands were from intrastrand AT exciplexes or interstrand processes, the mid-IR transient spectra of (dAdT)_9·(dAdT)_9 in Figures 5.14 and at elevated temperatures in Figure 5.9 were compared. In solution at room temperature, self-complementary (dAdT)_9·(dAdT)_9 forms a stable duplex structure with base stacking and pairing; however, at elevated temperatures as discussed in the previous
section, base pairing is significantly disrupted while base stacking interactions are maintained. The positive bands at 1630 and 1640 cm\(^{-1}\) are not observed in the spectra at elevated sample temperatures. This indicates that these transient bands are not due to intrastrand exciplex states, which would be present in these single strands even though base pairing is disrupted, but a possible marker for an interstrand process such as PCET.

The assignment of the bands at 1630 and 1640 cm\(^{-1}\) to proton transfer is supported by the steady-state FTIR spectrum of 1-methyladenosine-\(d_2\). 1-Methyladenine has the same imino tautomeric form expected for adenine following double-proton transfer. The FTIR spectrum of 1-methyladenosine-\(d_2\) in D\(_2\)O solution at room temperature has a strong band at 1648 cm\(^{-1}\) and a second weaker band at 1556 cm\(^{-1}\).\(^{17}\) The spectral window in Figure 5.14 does not extend down to 1556 cm\(^{-1}\), but the band at 1640 cm\(^{-1}\) is at a similar frequency to that of 1-methyladenosine-\(d_2\). Future experiments will probe at lower frequencies to determine whether a positive transient band is also present around 1556 cm\(^{-1}\).

Double proton transfer (DPT) and single proton transfer (SPT) reactions in neutral AT DNA bases has been the subject of extensive theoretical studies.\(^{18-22}\) A schematic for DPT in AT base pairs is shown in Figure 5.16. DPT is more favorable the SPT in neutral AT base pairs, however, the energy barrier for DPT is too high to be a favorable reaction pathway. Excited-state calculations predict barrierless proton transfer from excited interstrand CT states to the ground state in isolated AT base pairs in the gas phase.\(^{23,24}\) However, in aqueous solution, the energy of the PT state increases, making PT unfavorable.\(^{23}\) As discussed in Chapter 3 for GC base pairs, AT radical base pairs have a
lower barrier for proton transfer. For an exciplex state with strong charge transfer character, as proposed for the alternating (dAdT)$_9$·(dAdT)$_9$, the adenine would have significant cationic character and the thymine significant anionic character, essentially generating a radical cation and anion base pair. Bertran et al reported the SPT reaction in the AT radical cation is a slightly endothermic reaction (1.2 kcal/mol) with a low energy barrier of 1.6 kcal/mol. DPT is less favorable with a slightly larger energy barrier of 6.45 kcal/mol. Proton transfer in the AT radical anion base pair was predicted to be energetically unfavorable. Calculations including hydration effects are of interest for this system, as recent calculation on the GC radical cation showed a lowering of free-energy change and activation energy on the inclusion of water molecules. A recent theoretical study by Kinz-Thompson and Conwell on a AT radical cation pair embedded in a AT oligomer in solution predicted that interstrand proton transfer occurs at low temperatures (< 225 K).

5.4 Conclusions

Mid-IR transient absorption measurements were carried out on a series of A- and T-containing oligonucleotides. Possible marker bands for the AA-excimer state in d(A)$_{18}$ were observed at 1570 and 1617 cm$^{-1}$. A pair of strong positive bands are observed at 1630 and 1640 cm$^{-1}$ in the alternating (dAdT)$_9$·(dAdT)$_9$, but not in (dA)$_{18}$, (dT)$_{18}$, and (dAdT)$_9$·(dAdT)$_9$. The absence of these bands in (dAdT)$_9$·(dAdT)$_9$ at elevated sample temperatures, where base pairing is disrupted but substantial stacking remains, supports the hypothesis that these bands arise from interstrand processes. These positive bands are
proposed to be marker bands for proton transfer in \((\text{dAdT})_9\cdot(\text{dAdT})_9\). Additional measurements are underway to confirm this assignment.

References


Figure 5.1. Mid-IR transient absorption spectra of (dA)$_{18}$ in 100 mM phosphate D$_2$O buffer at select time delays following 270 nm excitation.
Figure 5.2. Difference spectra of FTIR and TRIR bleach signals for (dA)$_{18}$ in 100 mM phosphate D$_2$O buffer.
Figure 5.3. Kinetic signals at 1629 cm$^{-1}$, 1578 cm$^{-1}$, 1550 cm$^{-1}$ of (dA)$_{18}$ in 100 mM phosphate D$_2$O buffer following excitation at 270 nm. The solid line is best-fit curve to the data determined using the AICc fitting strategy.
Figure 5.4. Spectra for (dA)$_{18}$ in 100 mM phosphate D2O buffer at a) steady-state, b) 5 ps, c) 30 ps, and d) 500 ps. Solid lines are fits of the data using multiple Lorentzians; dashed lines are the individual components of the Lorentzian fits.
Figure 5.5. Mid-IR transient absorption spectra of (dT)$_{18}$ in 100 mM phosphate D$_2$O buffer at select time delays following 270 nm excitation.
Figure 5.6. Kinetic bleach signals at 1630 cm$^{-1}$, 1663 cm$^{-1}$, 1695 cm$^{-1}$ of (dT)$_{18}$ in 100 mM phosphate D$_2$O buffer following excitation at 270 nm. The solid line is best-fit curve to the data determined using the AICc fitting strategy.
Figure 5.7. Spectra for (dT)$_{18}$ in 100 mM phosphate D$_2$O buffer at a) steady-state, b) 5 ps, and c) 30 ps. Solid lines are fits of the data using multiple Lorentzian; dashed lines are the individual components of the Lorentzian fits.
Figure 5.8. Difference spectra of FTIR and TRIR bleach signals for (dT)$_{18}$ in 100 mM phosphate D$_2$O buffer.
Figure 5.9. Mid-IR transient absorption spectra of (dA)$_{18}$·(dT)$_{18}$ and (dAdT)$_9$·(dAdT)$_9$ at elevated sample temperatures in 100 mM phosphate D$_2$O buffer with 0.25M NaCl at select time delays following 270 nm excitation. The dashed lines are the inverted FTIR.
Figure 5.10. Kinetic bleach signals for \((dA)_{18} \cdot (dT)_{18}\) and \((dAdT)_{9} \cdot (dAdT)_{9}\) at elevated sample temperatures following excitation at 270 nm. The solid line is best-fit curve to the data determined using the AICc model. Fit amplitude and lifetimes are given in Table 5.2.
Figure 5.11. Spectrum of single-stranded \((dA)_{18}(dT)_{18}\) at 5 ps time delay fit to a sum of Lorentzians. Dashed lines are the individual components of the Lorentzian fit. The fit lacks a needed positive offset but shows the approximate location of bleach bands.
Figure 5.12. Room temperature mid-IR transient absorption spectra of (dA)$_{18}$⋅(dT)$_{18}$ in 100 mM phosphate D$_2$O buffer with 0.25M NaCl at select time delays following 270 nm excitation.
Figure 5.13. Kinetic signals at 1624, 1641, 1663 and 1700 cm$^{-1}$ of (dA)$_{18}$-(dT)$_{18}$ in 100 mM phosphate D$_2$O buffer at room temperature following excitation at 270 nm. The solid lines are best-fit curves to the data determined using the AICc fitting strategy. Fit amplitudes and lifetimes are given in Table 5.3.
Figure 5.14. Room temperature mid-IR transient absorption spectra of (dAdT)$_9$·(dAdT)$_9$ in 100 mM phosphate D$_2$O buffer with 0.25M NaCl at select time delays following 270 nm excitation.
Figure 5.15. Kinetic signals at 1586, 1619, 1633, 1647 and 1669 cm$^{-1}$ of (dAdT)$_9$-(dAdT)$_9$ in 100 mM phosphate D$_2$O buffer at room temperature following excitation at 270 nm. The solid lines are best-fit curves to the data determined using the AICc fitting strategy. Fit amplitudes and lifetimes are given in Table 5.3.
Figure 5.16. A schematic for double-proton transfer (DPT) in Watson-Crick AT base pairs.
Table 5.1. Best-fit parameters for probe wavenumbers shown in Figure 5.3 and 5.6. Italicized values in each column were globally linked during fitting.

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Table 5.2. Best-fit parameters for transient mid-IR signals of AT-DNA at elevated temperatures for probe wavenumbers shown in Figure 5.10. Italicized values in each column were globally linked during fitting.

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Table 5.3. Best-fit parameters for transient mid-IR signals of duplex AT DNA at room temperature shown in Figure 5.13 and 5.15. Italicized values in each column were globally linked during fitting.

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<th>$\tau_1$ / ps</th>
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<th>$\tau_2$ / ns</th>
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<th>$\tau_2$ / ns</th>
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Chapter 6: Selective Excitation Studies of d(G₃A₄G₃)

6.1. Introduction

There is interest in whether excitation energy deposited at one site in DNA can transfer to another site within a strand. Higher rates of photodamage to specific DNA sequences has been proposed to result from the transfer of energy from sites of initial excitation to pyrimidine-rich sites that are susceptible to photodamage within a strand.¹⁻⁵ Understanding energy transfer in DNA can provide insight into site-specific DNA damage and possible uses of DNA for molecular electronic devices.

One way energy transfer in DNA has been studied has been to insert a fluorescent reporter molecule, such as 2-aminopurine (2AP), that acts as an energy trap into the DNA and monitor emission of the molecules as a function of excitation.⁶,⁷ Nordlund et al. showed that singlet energy transfer occurs between DNA bases and 2AP in a DNA decamer.⁵ The efficiency of energy transfer was found to be dependent on sequence and temperature. A subsequent study on a range of DNA oligonucleotides with embedded 2APs reported that energy transfer was more efficient in A-tracts and has an average transfer distance of 4 adenine bases or less.⁷ Another approach to studying energy transfer in DNA strands used by Ge and Georghiou was to selectively excite different nucleobases within a strand and monitor emission as a function of excitation
Ge and Georghiou monitored the fluorescence spectra of alternating\textsuperscript{8,9} and non-alternating\textsuperscript{8} AT polymers after exciting both A and T at bases at 265 nm or just the T bases at 293 nm. Electronic energy transfer was reported to occur from T to A bases in the alternating system, but not between strands in the non-alternating system\textsuperscript{8,9}.

The selective excitation experiments by Ge et al.\textsuperscript{8,9} and more recently by Takaya et al.\textsuperscript{10} used the fact that the UV absorption spectrum of adenine is unique in that it is blue shifted relative to the absorption spectra of the other DNA bases (C, T, and G) (Figure 6.1). Takaya et al. used selective excitation to study exciplex formation in ApC dinucleosides.\textsuperscript{10} Ground-state recovery signals of the dinucleoside ApC were compared following 266 nm excitation, where A and C bases are excited, and 290 nm excitation, where C bases were selectively excited. From this study, it was concluded that energy does not transfer from the excited C bases to unstacked A bases based on the absence of a fast decay component in signals at 290 nm excitation. The long-lived signals observed were assigned to excited states formed in the stacked ApC bases.

In the studies reported in this Chapter, we are combining for the first time selective excitation techniques with structurally sensitive time-resolved vibrational spectroscopy in order monitor excitation energy on specific bases. In electronic spectroscopy, it is difficult to determine the contribution of individual types of bases to the spectra.\textsuperscript{11} In contrast, in model DNA systems, it is possible to have distinct vibrational bands assigned to the individual DNA bases. For this study, the sequence d(G\textsubscript{3}A\textsubscript{4}G\textsubscript{3}) was selected because at an excitation wavelength of 270 nm, both the A and G bases are excited; while at the longer wavelength of 290 nm, the G bases are primarily
excited, as shown in Figure 6.1. In addition, the infrared spectrum of d(G₃A₄G₃) has well-separated vibrational bands for the A and G bases in the 1600 to 1700 cm⁻¹ region that can be monitored as a function of excitation wavelength and provide information on were the excitation energy is in the strand. These properties will allow us to selectively excited A and G bases and monitor specific vibrational bands associated with each type of base as a function of time. The preliminary results of selective excitation experiments on d(G₃A₄G₃) are reported here.

6.2. Results

6.2.1 Steady-state FTIR and Absorption Spectra

Figure 6.1 shows the steady-state UV absorption spectra of GMP, AMP, and d(G₃A₄G₃) in phosphate buffer. The spectra of GMP and AMP have been scaled to reflect the relative quantity of each base in the oligomer (ie. 6 guanines and 4 adenines). The purple curve is the sum of the scaled monomer spectra; the green curve is the actual spectra of d(G₃A₄G₃) in phosphate buffer. The spectrum of the oligomer has a decreased absorption at the absorption maximum of 260 nm and has a red-shifted tail extending out to 300 nm. At 270 nm, A and G bases have similar absorption cross sections (e₂₇₀(G) = 9,400 and e₂₇₀(A) = 11,300). At wavelengths longer than 285 nm, A absorbs negligibly compared to G. At 290 nm, G and A bases have calculated absorption cross section of e₂₉₀(G) = 2,400 e₂₉₀(A) = 0, respectively.

The FTIR spectrum of d(G₃A₄G₃) from 1600 to 1700 cm⁻¹ is shown in the top panel of Figure 6.2 (dashed line). Two vibrational bands are present in this spectral
region: a band at 1626 cm\(^{-1}\) assigned to the ring vibrations in adenine and an intense band at 1673 cm\(^{-1}\) has contributions from the C=O and C5=C6 carbonyl stretches of guanine.\(^{13}\) Hereafter, the band at 1626 cm\(^{-1}\) will be referred to as the A band and the band at 1673 cm\(^{-1}\) will be the G band.\(^{13}\) The amplitude of the A-band is approximately a quarter of that of the G-band.

6.2.2. UV-pump / UV-probe Transient Absorption Signals

Figure 6.3 shows the transient absorption signals of d(G\(_3\)A\(_4\)G\(_3\)) at 250 nm following 266 nm and 290 nm excitation. Identical signals are observed at both pump wavelengths. The signals are well fit to three exponentials, with lifetimes of 3.7 ± 0.5, 48 ± 21 and 382 ± 100 ps. Approximately half of the signal amplitude comes from the fastest component. It should be noted that a 2 exponential fit gives lifetimes of 4.5 ± 0.4 and 129 ± 19 ps. The 129 ps lifetime is similar to reported lifetimes for AA and AG exciplex states,\(^{10,14-16}\) but 3 exponentials give a better fit and lower AICc value (Section 2.6). Lifetimes and amplitudes for the two and three exponentials fits are compared in Table 6.1.

6.2.3. UV-pump / Mid IR-probe Transient Absorption Spectra

The TRIR spectra of d(G\(_3\)A\(_4\)G\(_3\)) obtained at selected time delays from 1 ps to 3 ns following excitation at 270 and 292 nm are shown in Figure 6.2. The amplitude of the bleach band 1626 cm\(^{-1}\) is dependent on pump wavelength. At an excitation wavelength of 270 nm, a strong transient bleach band is observed at 1674 and a weaker band at 1626 cm\(^{-1}\). At 290 nm excitation, a bleach band of similar amplitude is observed at 1674 cm\(^{-1}\) and a significantly weaker band is present at 1626 cm\(^{-1}\). The amplitudes of the A- and G-
bands were compared at 10 ps when the hot ground state has recovered to the ground state. At 10 ps, the A-band has approximately 33% of the amplitude of the G-band following excitation at 270 nm. The relative amplitude of these two bands matches that of the FTIR spectrum. At 290 nm excitation, the A-band has approximately 10% of the amplitude of the G-band at 10 ps. An underlying flat positive band stretches from at least 1600 and 1645 cm\(^{-1}\). At long times, the 290 nm spectrum is nearly recovered to the ground state, while the 270 nm spectrum is not fully recovered especially the bleach at 1674 cm\(^{-1}\).

6.2.4. UV-pump / mid-IR probe Transient Absorption Kinetic Signals

The transient signals and fits at 1626, 1645, and 1674 cm\(^{-1}\) are shown in Figure 6.4. The signals were normalized in Figure 6.5 to highlight similar decay components. The kinetics of the positive band at 1645 cm\(^{-1}\) is unaffected by the excitation wavelength, decaying with lifetimes of 11 ± 3 ps at both excitation wavelengths. At 270 nm, a small offset is observed at long times. The bleach band at 1674 cm\(^{-1}\) is well fit to three exponentials at both 270 and 290 nm. At 270 nm excitation, the bleach decays with lifetimes of 3.9 ± 0.7, 33 ± 4, 444 ± 180 ps and a significant offset. Slight differences are observed at 290 nm; the band decays with lifetimes of 3.9 ± 0.7, 33 ± 4, 1120 ± 150, and no offset. Fit parameters are listed in Table 6.1.

The 270 and 290 nm excitation bleach signals for of the A-band at 1626 cm\(^{-1}\) differ at early times but match after 10 ps. The scaled signals of 1626 cm\(^{-1}\) in Figure 6.5 highlight the differences at early time following 270 and 290 nm excitation. At 270 nm, the bleach signal is well fit to two exponentials with lifetimes of 4.9 ± 1.4 and 207 ± 100
ps. At 290 nm, the fastest component is absent. The bleach signal is well fit to three exponentials with lifetimes of $10 \pm 9$, $11 \pm 3$, and $207 \pm 100$ ps. The $11 \pm 3$ ps has a positive amplitude and decays at the same rate as the underlying positive transient band. After 10 ps, the signal at 290 nm has approximately a third of the amplitude of the signal excited at 270 nm (Figure 6.3).

6.3. Discussion

6.3.1. The Oligomer $d(G_3A_4G_3)$ is Single-stranded in Solution

G-rich oligonucleotides frequently self-associate to form higher-ordered structures called G-quadruplexes. The C6=O6 carbonyl stretch of guanine has been shown to be sensitive to base pairing, shifting from 1668 cm$^{-1}$ to higher wavenumbers when base pairing is present. Dagneau et al. reported that addition of salt to $d(G_3A_4G_3)$ induces self-association and results in a shift of the 1673 cm$^{-1}$ band to 1681 cm$^{-1}$. In the absence of salt, no shift was observed indicating that the strand is unassociated in solution. For this experiment, $d(G_3A_4G_3)$ was prepared in phosphate buffer without salt. The FTIR spectrum shown in Figure 6.2 was recorded before transient absorption measurements. The position of the G band at 1673 cm$^{-1}$ indicates that $d(G_3A_4G_3)$ is unassociated and single-stranded in our experimental conditions.

6.3.2. Selective Excitation of $d(G_3A_4G_3)$ Monitored by Transient IR Spectroscopy

The transient signals for the A-band at 1626 cm$^{-1}$ and G-band at 1674 cm$^{-1}$ following 270 nm excitation (Figure 6.4) are similar to those reported for A-containing and G-containing homo-DNA using UV/UV and UV/mid-IR spectroscopy. The A-
band has a biexponential decay with lifetimes of 4.9 ± 1.4 and 207 ± 100 ps. As discussed in Chapter 5 for (dA)$_{18}$, the 4.9 ± 1.4 ps decay component is similar to the vibrational cooling times observed in AMP and poly(A) in UV/mid-IR transient absorption experiments,\textsuperscript{19,20} but slower than the 2.9 ps reported for AMP in UV/UV studies in D$_2$O.\textsuperscript{19} The 207 ± 100 ps lifetime is within experimental uncertainty the same as the lifetimes reported for the AA excimer state.\textsuperscript{10,14,15}

McGovern \textit{et al.} studied GMP and polyG in D$_2$O using UV/mid-IR transient absorption spectroscopy.\textsuperscript{21} Biexponential decays were reported for both the monomer and polymer solutions, with a fast 4-5 ps component and a long-lived component. Lifetimes of 49 ± 9 ps and 37 ± 9 ps were reported for the long-lived components in monomer and polymer, respectively, and assigned to excimer states formed in stacked G-tetrads.\textsuperscript{21} The transient signal for the G-band in Figure 6.4 is well fit to three exponentials with lifetimes of 3.9 ± 0.7, 33 ± 4, and 400 - 1200 ps depending on pump wavelength (Table 6.1). The 3.9 ± 0.7 component matches the lifetime of the fast component reported for GMP\textsuperscript{21} and is assigned to monomer-like decay. The 33 ± 4 ps intermediate lifetime is similar to the lifetime reported for GG excimer states formed in G-tetrads.\textsuperscript{21} The 400 - 1200 ps component is not observed in GMP or polyG.

The transient absorption signals in Figure 6.2 show a significant change in the bleach amplitude of the A-band with different pump wavelengths. Two bleach bands are observed at 1626 and 1674 cm$^{-1}$ following 270 and 290 nm excitation. The frequencies of these transient bands match up with the ground state IR bands for A and G and are assigned to ground-state bleach bands. At 270 nm excitation, the relative amplitudes of
the A- and G- bands match those of the ground-state IR bands. In contrast, following excitation at 292 nm, a strong bleach is observed at 1674 cm\(^{-1}\) and a weak bleach is observed at 1626 cm\(^{-1}\). The signals at 1674 cm\(^{-1}\) have similar dynamics for the two pump wavelengths, with a larger offset seen at long times following 270 nm excitation. This residual bleaching at 3 ns after 270 nm excitation could result from photodegradation and increased D\(_2\)O heating\(^{22}\) due to the greater pump intensity.

Comparing the early times spectra in Figure 6.2 shows that excitation at 292 nm results in the localized excitation of guanines in the G-tracts and not the adenines in the A-tracts in d(G\(_3\)A\(_4\)G\(_3\)). As we expect the G-tracts to have a lower-energy excited state, it is not surprising that a higher-lying A excited-state is not populated. It also shows that there is no common low energy state in this system. Consecutive G bases are proposed to act as hole traps for charge transfer in DNA.\(^{23,24}\) The early decay of the A-band at 1626 cm\(^{-1}\) matches the decay time of the hot ground stand bands (Figure 6.4). The normalized signals for 1626 cm\(^{-1}\) in Figure 6.4 show that it takes about 10 ps to fully develop the bleach of the A band. The initial growth of the 1626 cm\(^{-1}\) band after excitation is likely due to the decay of the stronger underlying hot ground-state absorption band. After 10 ps, the decay of the 1626 cm\(^{-1}\) following 270 and 290 nm excitation match although the amplitude at 290 nm is less than half of that for 270 nm excitation. The similar decays indicates that there is no energy transfer between A and G bases in d(G\(_3\)A\(_4\)G\(_3\)) after 10 ps. At earlier delay times, it is difficult to determine if there is energy transfer due to the underlying hot ground state dynamics.
Although A has negligible absorption at 290 nm, a weak bleach band is present at 1626 cm\(^{-1}\) following 290 nm excitation. This weak bleach could be due to weak absorption of adenines in the A-tract at 290 nm; however, the amplitude of this band is stronger than expected given the negligible absorption cross section of A at 290 nm. A-tracts, like the A\(_4\) segment in this sequence, exhibit a red tail in their absorption spectra due to excitonic interactions. It seems more likely that we are exciting stacked A bases, which have stronger absorption at 290 nm. This also explains why there is no fast decay component from unstacked A bases at 290 nm, but only a long-lived component assigned to excited-states formed in stacked bases. Another possibility for the weak bleach at 1626 cm\(^{-1}\) is that we are forming AG exciplex states at the border between the G- and A-tracts. Formation of an exciplex state would result in excitation energy shared between both the A and G bases. This could also explain why the band at 1626 cm\(^{-1}\) at 290 nm excitation has 1/3 the amplitude of the band at 270 nm excitation even though the A bases have negligible absorption at this pump wavelength.

6.4. Conclusions

This preliminary study shows that we have localized excitation in a single-stranded system composed of A and G tracts following excitation at 290 nm. This work is the beginning of future studies using selective excitation coupled with TRIR spectroscopy to investigating energy transfer or excimer migration in DNA.
References


Figure 6.1. UV absorption spectra of the monomers GMP and AMP, the single-stranded d(G₃A₄G₃).
Figure 6.2. Mid-IR probe transient signals at select time delays for d(G$_3$A$_4$G$_3$) following 270 nm pump (left panel) and 290 nm pump (right panel).
Figure 6.3. Transient absorption signals at the probe wavelength of 250 nm and pump wavelengths of 266 nm (red) and 290 nm (blue). At 290 nm, the adenine bases have minimal UV absorption.
Figure 6.4. Mid-IR probe signals at select frequencies following 270 nm pump (closed circles) and 292 nm (open circles). Solid lines are best fits curves.
Figure 6.5. Scaled transient absorption signals at excitation wavelengths of 270 nm (solid) and 290 nm (dashed) and different probe wavenumbers.
Table 6.1. Best-fit parameters for duplex AT DNA shown in Figure 6.2 and 6.3. Bold values in each column were globally linked during fitting.

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<th>τ₁ / ps</th>
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*positive amplitude
Bibliography


150


(64) Inman, R. B. J. Mol. Biol. 1964, 9, 624-37.


