JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Ultrafast Excited-State Dynamics in Hexaethyleneglycol-Linked DNA Homoduplexes Made of A·T Base Pairs

Jinquan Chen,[†] Arun K. Thazhathveetil,[‡] Frederick D. Lewis,*[,‡] and Bern Kohler*[,†]

[†]Department of Chemistry and Biochemistry, Montana State University, P.O. Box 173400, Bozeman, Montana 59717, United States
[‡]Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, United States

Supporting Information

ABSTRACT: Double-stranded DNA conjugates with the sequence $(dA)_{10}\cdot(dT)_{10}$ and hexaethylene glycol linkers at one end (hairpin) or both ends (dumbbell) were studied in buffer solution by deep UV femtosecond transient absorption spectroscopy. These covalently constrained duplexes have greatly enhanced thermal stability compared to A·T duplex oligonucleotides that lack linkers. The conjugates eliminate the slipped-strand and end-frayed structures that form readily in unlinked $(dA)_n\cdot(dT)_n$ sequences, allowing the excited-state dynamics of stacked A·T base pairs to be observed without interference from structures with stacking or pairing defects. Transient absorption signals show that subpicosecond internal conversion to the electronic ground state takes place in addition to the formation of long-lived excited states having lifetimes of approximately 70 ps. Watson–Crick base-pairing slows the rate of vibrational cooling compared to monomeric bases or single-stranded DNA, possibly by reducing the total number of solute–solvent hydrogen bonds. Long-lived excited states in intact A·T base pairs decay several times more quickly than long-lived excited states observed in single-stranded $(dA)_n$ sequences. These results show that base-pairing can measurably affect nonradiative decay pathways in A·T duplexes.

Structural heterogeneity is a significant obstacle to mapping the various nonradiative decay pathways in double-stranded DNA. Recent experiments have shown that structural disorder profoundly influences the excited-state dynamics of single-stranded model compounds,[1] but effects in duplex DNA are less certain. Many of the duplex model systems studied to date are double-stranded structures containing just A and T bases and fewer than 20 base pairs.[2−4] However, the low thermal stability of such duplexes gives rise to a distribution of structures in room-temperature aqueous solution. Quite apart from the possible presence of single strands due to concentration-dependent hybridization, disorder in homoduplex structures can result from frayed ends and slipped strands (structures a and b in Figure 1). In addition, a single base can flip out of the helix, resulting in the loss of base-pairing and stacking. These and other structures may differ only modestly in free energy compared to a defect-free duplex with the maximum possible number of Watson–Crick base pairs.

In order to prevent structural heterogeneity from obscuring excited-state dynamics, DNA model compounds with well-defined conformational states are highly desirable. In this study, hexaethyleneglycol-linked DNA dumbbell and hairpin conjugates containing 10 A·T base pairs (structures c and d in Figure 1) were chosen because these systems adopt a B-DNA structure but have much higher thermal stability than unlinked duplexes.[5] The dumbbell used in this study has a melting temperature of approximately 80 °C compared to 40 °C for a bimolecular duplex with the same number of base pairs. The results obtained on these highly ordered systems reveal a number of important new insights into excited-state dynamics in duplex DNA.

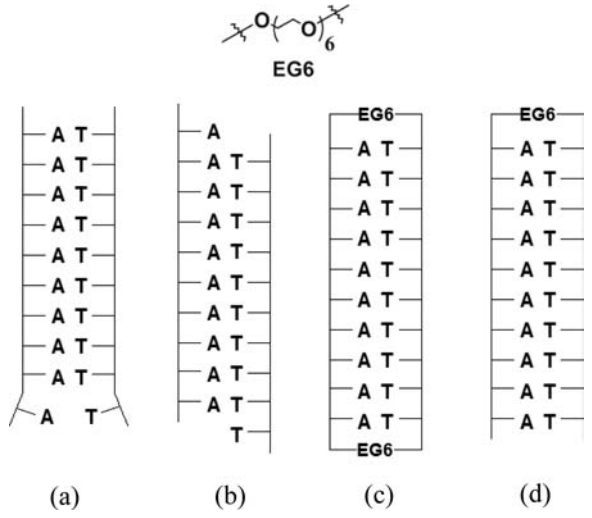

Figure 1. A·T DNA homoduplexes with (a,b) and without (c,d) stacking and pairing defects: (a) end-frayed duplex, (b) slipped-strand duplex, (c) ordered dumbbell, and (d) hairpin structures with hexaethyleneglycol linkers.

The non-chromophoric hexaethyleneglycol linker has a great effect on conformation by preventing strand slippage and end-fraying, but it does not perturb the electronic structure of the duplex.[5] UV/vis absorption spectra of the dumbbell and hairpin agree within experimental uncertainty and are almost identical to the absorption spectrum of an unlinked $(dA)_n\cdot(dT)_n$ duplex (Figure S1). UV/UV pump–probe measurements on the single strands $(dA)_{10}$ and $(dT)_{10}$ having a hexaethyleneglycol moiety appended to the 5′ end agree with signals from unmodified $(dA)_{18}$ and $(dT)_{18}$ oligonucleotides (Figure S2). This agree-

Received: May 16, 2013
Published: July 1, 2013

   dx.doi.org/10.1021/ja4049459 | J. Am. Chem. Soc. 2013, 135, 10290−10293

ment confirms that the linker does not alter excited-state dynamics in the single strands.

Transient absorption signals were recorded at a pump wavelength of 265 nm and a probe wavelength of 250 nm for the dumbbell and hairpin conjugates at several temperatures (Figure 2). The strong bleaching ($\Delta A < 0$) seen at 250 nm reveals all time scales needed to repopulate the electronic ground state.[1,4]

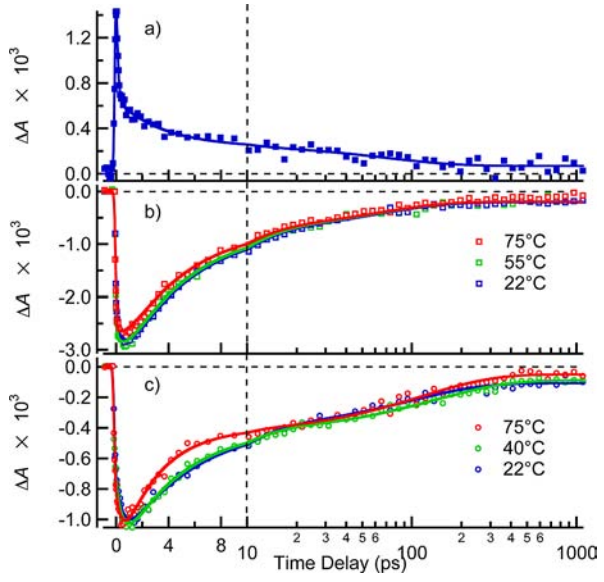

Figure 2. Transient absorption signals (pump 265 nm) from (a,b) dumbbell and (c) hairpin A·T conjugates in buffer solution at the indicated temperatures. The probe wavelength for the room-temperature transient in (a) is 350 nm. All other transients were recorded at a probe wavelength of 250 nm.

The dumbbell signals agree within experimental uncertainty at all three temperatures. They increase slightly in the first several hundred femtoseconds after time zero, requiring a subpicosecond decay component with positive amplitude. After the maximum bleach signal is reached, the signal decreases by about 60% with a time constant of 4.7 ± 0.5 ps; the remaining signal decays with a longer time constant of 62 ± 17 ps (all fitting parameters are summarized in Table S1).

The hairpin signals also recover toward zero on two distinct time scales, immediately after a weak subpicosecond component also noted in the dumbbell. Best-fit time constants for the room-temperature hairpin signal agree with those determined for the dumbbell within experimental uncertainty. As temperature is increased above the hairpin melting temperature, the bleach recovers more rapidly in the first 10 ps, but then more slowly afterward (Figure 2c).

The signals in Figure 2 indicate that the dumbbell maintains a rigid, base-paired structure up to 75 °C, while the hairpin signals gradually acquire the characteristics of signals from the separate A and T strands (Figure 3). Temperature-dependent CD spectra (Figure S3) confirm that base-pairing is maintained in the dumbbell at the highest temperatures. On the other hand, the CD spectrum of the hairpin becomes increasingly similar to that of a $(dA)_n$ oligonucleotide[6] at temperatures above 60 °C. This is a clear indication that significant base stacking remains in the denatured A strand despite the high temperature.

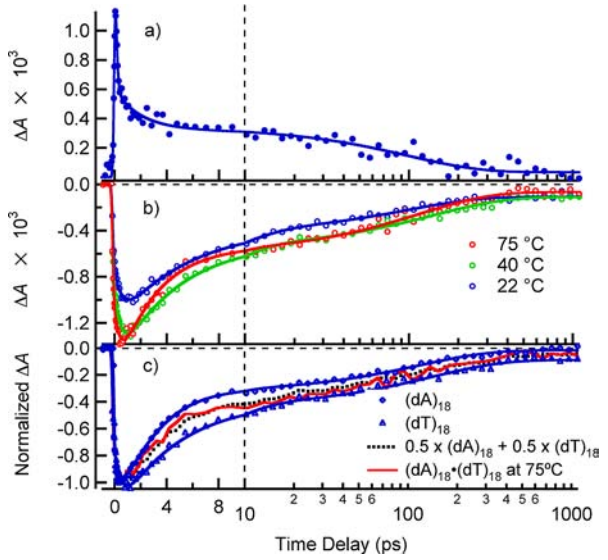

Figure 3. Transient absorption signals (pump 265 nm) from an unlinked $(dA)_{18}\cdot(dT)_{18}$ homoduplex at a probe wavelength of 350 nm (a, room temperature) and 250 nm (b, indicated temperatures). Panel c compares the 250 nm probe signal from the homoduplex at 75 °C (solid red line) with the linear combination of signals from $(dA)_{18}$ and $(dT)_{18}$ recorded at room temperature.

The large amplitude of the 4.7 ps decay component suggests that this signal arises from population transfer between electronic states and is not due to small shifts in the spectra of excited-state molecules induced by vibrational relaxation or solvation. This component is assigned to vibrational cooling following ultrafast internal conversion—an assignment that is confirmed by the transient signal recorded for the dumbbell at a probe wavelength of 350 nm (Figure 2a). This wavelength falls within an excited-state absorption band seen for $(dA)_n$ oligonucleotides.[7,8] The pronounced subpicosecond decay at 350 nm is assigned to ultrafast internal conversion to the electronic ground state. The rapid return of the excited-state population to the ground electronic state then initiates the vibrational cooling response that is monitored at 250 nm.

The subpicosecond decay component seen at 250 nm could reflect the time needed to repopulate the electronic ground state. Alternatively, there could be an ultrafast contribution to the vibrational cooling signal.[10] Disentangling the overlapping signal contributions from electronic and vibrational relaxation is extremely challenging and would require higher time resolution and monitoring of specific vibrations. As this is not our objective in this study, we will not discuss the subpicosecond signals other than to note that they are consistent with ultrafast relaxation to the electronic ground state.

Interestingly, the 4.7 ps lifetime seen at room temperature in the dumbbell and hairpin is twice as long as the 2.7 ps vibrational cooling lifetime observed in $(dA)_n$ single strands[1] or the 2.38 ± 0.12 ps lifetime reported for single-stranded $(dT)_{18}$.[4] A recent femtosecond UV pump/mid-IR probe study of several xanthine derivatives has shown that vibrational cooling rates increase with the number of solute−solvent hydrogen bonds.[9] In duplex DNA, base-pairing between A and T prevents two N−H bonds from forming hydrogen bonds with water molecules, and we propose that this slows vibrational energy transfer from a hot base to its environment. The complementary base takes the place of one or more water molecules, and vibrational cooling consequently slows down as observed

dx.doi.org/10.1021/ja4049459 | J. Am. Chem. Soc. 2013, 135, 10290−10293

for single nucleobases in organic solvents.[10] The decrease in the vibrational cooling lifetime for the hairpin from 4.7 ps at 22 °C to 3.5 ps at 75 °C (Table S1) is consistent with accelerated vibrational cooling as the base pairs are disrupted.

Slower vibrational cooling in duplex DNA has not been discussed before, but review of previously reported transient absorption measurements reveals a pattern of generally slower cooling lifetimes in duplex DNAs than in single strands or base monomers. A vibrational cooling lifetime of 3.4 ps was reported for a mixed-sequence oligonucleotide containing 11 base pairs.[11] Lifetimes of 3−6 ps were observed for various duplex oligonucleotides possessing GC base pairs.[12]

The subpicosecond decay component seen in the dumbbell at 350 nm and the vibrational cooling signatures seen in both the dumbbell and hairpin structures at room temperature reveal that ultrafast internal conversion to the ground state is an accessible decay pathway for excited states in regions of DNA with stacked base pairs. In the past, the ubiquitous decay component of a few picoseconds that is seen in transient bleach signals from DNA single and double strands was suggested to arise from excited states localized on unstacked bases.[1,4] Such bases are envisioned to be more distant from neighboring bases and to have greater solvent exposure, two factors that are likely to reduce the interbase electronic couplings needed to rapidly populate long-lived excited states in competition with monomer-like nonradiative decay.

In a low-melting A·T homoduplex, unstacked bases can be found in disordered single-stranded regions present in frayed ends or slipped-strand duplexes (Figure 1). However, the high-melting conjugates studied here are expected to have few, if any, stacking defects, particularly near room temperature. The observation of subpicosecond internal conversion in a fully stacked A·T system thus indicates that the formation of long-lived excited states is not the exclusive decay channel for excitations in duplex DNA in agreement with the conclusion from a study of a mixed-sequence duplex.[11]

Several authors of computational studies have suggested that monomer-like (i.e., subpicosecond) decay is possible alongside slower decay pathways in stacked bases.[13−16] Ultrafast localization of an initially delocalized exciton on a single base is thought to be the initial step in this process. This picture is supported by calculations showing that excited-state minima are little changed when a nucleobase is incorporated in a double strand.[14]

Our results demonstrate monomer-like decay only in a double-stranded context, but calculations suggest that ultrafast localization and decay also occur in single strands.[13] Su et al. recently estimated that the fraction of stacked nucleotides increases from 46% in $(dA)_2$ to 71% in $(dA)_{18}$ by assuming that only unstacked nucleotides contribute to the short-lived signal component.[1] Monomer-like decay in single-stranded base stacks could explain why these values are systematically lower than other stacking estimates.[6]

Other computational studies have addressed how excited-state deactivation by a single base, which is treated fully quantum mechanically, is affected by electrostatic and non-covalent interactions in a double helix, which is treated at the level of molecular mechanics.[17−20] On the basis of static calculations, it was proposed that steric hindrance could slow nonradiative decay by a base-localized excited state in DNA to the hundreds of picoseconds time scale.[18] On the other hand, quantum dynamical calculations have predicted that lifetimes are increased only to the low picosecond range.[17,19,20] The transient signal at 350 nm requires three exponentials for a suitable fit. The shortest lifetimes of 100 fs and 3.4 ps may reflect the time needed for localization and subsequent internal conversion, or they could indicate that monomer-like relaxation unfolds on two time scales.

The approximately 70 ps lifetime observed in both the dumbbell and hairpin at room temperature is shorter than the lifetime of 101 ± 6 ps reported previously for an unlinked $(dA)_{18}\cdot(dT)_{18}$ duplex at the same probe wavelength of 250 nm.[4] Furthermore, the 4.7 ps decay component observed here agrees poorly with the 2.46 ± 0.10 ps reported in ref 4. This disagreement led us to reinvestigate transient absorption by a $(dA)_{18}\cdot(dT)_{18}$ duplex. The results (Figure 3) agree reasonably well with the room-temperature transients for the dumbbell and hairpin structures. As shown in Figure 3, at the highest temperature of 75 °C, the bleach recovery signal at 250 nm is well reproduced by the average signals measured for separated $(dA)_{18}$ and $(dT)_{18}$ strands. In other words, melting of the duplex structure is accompanied by a reduction in the vibrational cooling lifetime and an increase in the lifetimes of long-lived excited states, which become more similar to the long-lived states due to stacked bases in the dA strand, or due to $^1n\pi^*$ states[21] in the dT strand.

The transients reported in ref 4 for $(dA)_{18}\cdot(dT)_{18}$ are now seen to have been obtained at an elevated temperature at which the duplex would have been substantially denatured. Highly efficient nonradiative decay by DNA means that nearly every absorbed photon is converted into heat. A spinning cell was used in ref 4, but slow diffusion of molecules out of the pumped region[22] and tight focusing of the pump beam can lead to temperature jumps of more than 20 °C, as we plan to discuss in a later publication.

The flow cell used in this study completely eliminates laser-induced melting, allowing the excited-state dynamics of stacked A·T base pairs to be accurately characterized for the first time. Base-pairing shortens the lifetime of long-lived excited states from 100−200 ps in single-stranded $(dA)_n$[1] to about 70 ps. This decrease is counter to the increase that might have been expected in the more sterically hindered environment of the double helix.[18] Although the 70 ps lifetime is more similar to the lifetimes observed in single-stranded stacks of adenine bases than to the ultrafast decay suggested to occur in A·T base pairs,[23] the results described here do indicate non-negligible effects due to base-pairing on nonradiative decay in double-stranded DNA. The possibility that interstrand proton transfer could play a role in the deactivation mechanism, as suggested previously for GC duplexes,[12] is intriguing but will require further experimental and theoretical study.

In summary, excited states in hexaethyleneglycol-linked DNA hairpin and dumbbell duplexes containing 10 A·T base pairs decay to the electronic ground state on two distinct time scales. A 4.7 ps decay component is assigned to vibrational cooling, which occurs a factor of 2 more slowly than in single-stranded DNA. Base-pairing decreases the number of base−water hydrogen bonds, leading to slower energy dissipation. Ultrafast internal conversion to the electronic ground state is a competitive decay channel for excitations in stacked A·T base pairs that coexists with a second decay channel with a characteristic lifetime of about 70 ps. Nonradiative decay via the latter channel occurs about twice as rapidly as in single-stranded $(dA)_n$ sequences, revealing a modest quenching effect due to base-pairing that warrants further experimental and computational study.

■ ASSOCIATED CONTENT

Supporting Information

Experimental methods, supplementary figures showing absorption spectra, temperature-dependent CD spectra, single-strand kinetics, and a table of best-fit parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

kohler@chemistry.montana.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Funds from the U.S. National Science Foundation (CHE-1112560 to B.K.) and the U.S. Department of Energy, Office of Basic Energy Sciences (DE−FG02−96ER14604 to F.D.L.) are gratefully acknowledged.

■ REFERENCES

(1) Su, C.; Middleton, C. T.; Kohler, B. *J. Phys. Chem. B* **2012**, *116*, 10266.
(2) Markovitsi, D.; Sharonov, A.; Onidas, D.; Gustavsson, T. *ChemPhysChem* **2003**, *4*, 303.
(3) Markovitsi, D.; Onidas, D.; Gustavsson, T.; Talbot, F.; Lazzarotto, E. *J. Am. Chem. Soc.* **2005**, *127*, 17130.
(4) Crespo-Hernández, C. E.; Cohen, B.; Kohler, B. *Nature* **2005**, *436*, 1141.
(5) McCullagh, M.; Zhang, L.; Karaba, A. H.; Zhu, H.; Schatz, G. C.; Lewis, F. D. *J. Phys. Chem. B* **2008**, *112*, 11415.
(6) Olsthoorn, C. S. M.; Bostelaar, L. J.; De Rooij, J. F. M.; Van Boom, J. H.; Altona, C. *Eur. J. Biochem.* **1981**, *115*, 309.
(7) Kwok, W.-M.; Ma, C.; Phillips, D. L. *J. Am. Chem. Soc.* **2006**, *128*, 11894.
(8) Buchvarov, I.; Wang, Q.; Raytchev, M.; Trifonov, A.; Fiebig, T. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 4794.
(9) Zhang, Y.; Chen, J.; Kohler, B. *J. Phys. Chem. A* **2013**, DOI: 10.1021/jp4040002.
(10) Middleton, C. T.; Cohen, B.; Kohler, B. *J. Phys. Chem. A* **2007**, *111*, 10460.
(11) de La Harpe, K.; Kohler, B. *J. Phys. Chem. Lett.* **2011**, *2*, 133.
(12) de La Harpe, K.; Crespo-Hernández, C. E.; Kohler, B. *J. Am. Chem. Soc.* **2009**, *131*, 17557.
(13) Santoro, F.; Barone, V.; Improta, R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 9931.
(14) Santoro, F.; Barone, V.; Improta, R. *J. Am. Chem. Soc.* **2009**, *131*, 15232.
(15) Improta, R.; Barone, V. *Angew. Chem., Int. Ed.* **2011**, *50*, 12016.
(16) Improta, R. *J. Phys. Chem. B* **2012**, *116*, 14261.
(17) Nachtigallova, D.; Zeleny, T.; Ruckenbauer, M.; Muller, T.; Barbatti, M.; Hobza, P.; Lischka, H. *J. Am. Chem. Soc.* **2010**, *132*, 8261.
(18) Conti, I.; Altoè, P.; Stenta, M.; Garavelli, M.; Orlandi, G. *Phys. Chem. Chem. Phys.* **2010**, *12*, 5016.
(19) Lu, Y.; Lan, Z. G.; Thiel, W. *Angew. Chem., Int. Ed.* **2011**, *50*, 6864.
(20) Zeleny, T.; Ruckenbauer, M.; Aquino, A. J. A.; Muller, T.; Lankas, F.; Drsata, T.; Hase, W. L.; Nachtigallova, D.; Lischka, H. *J. Am. Chem. Soc.* **2012**, *134*, 13662.
(21) Hare, P. M.; Crespo-Hernández, C. E.; Kohler, B. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 435.
(22) Pan, Z. Z.; Chen, J. Q.; Schreier, W. J.; Kohler, B.; Lewis, F. D. *J. Phys. Chem. B* **2012**, *116*, 698.
(23) Perun, S.; Sobolewski, A. L.; Domcke, W. *J. Phys. Chem. A* **2006**, *110*, 9031.