Ultrafast Energy Transfer and Structural Dynamics in DNA

A. Trifonov,† M. Raytchev,† I. Buchvarov,† M. Rist,‡ J. Barbaric,‡ H.-A. Wagenknecht,*,‡ and T. Fiebig*,†

Eugene F. Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02467, and Chemistry Department, Technical University of Munich, Lichtenbergstr. 4, D-85747 Garching, Germany

Received: April 22, 2005; In Final Form: August 4, 2005

Ultrafast structural dynamics concomitant to excitation energy transfer in DNA has been studied using a pair of pyrene-labeled DNA bases. The temporal evolution of the femtosecond pump−probe spectra reveals the existence of two electronic coupling pathways, through-base stack and through-space, which lead to excitation energy transfer and excimer formation even when the labeled DNA bases are separated by one AT base pair. The electronic coupling which mediates through-base stack energy transfer is so strong that a new absorption band arises in the excited-state absorption spectrum within 300 fs. From the analysis of time-dependent spectral shifts due to through-space excimer formation, the local structural dynamics and flexibility of DNA are characterized on the picosecond and nanosecond time scale.

1. Introduction

Over the past decade, the structural[1−4] and electronic properties[5] of DNA have been the focus of intense research efforts. DNA is a complex medium with internal dynamics on time scales that span over 15 orders of magnitude (from 10^{-13} to 10^{2} s).[2,6] Various techniques have been applied to study DNA dynamics on the time scales of seconds to milliseconds,[4] nanoseconds, and subnanoseconds.[7,8] While slow dynamics are more easily related to biological function, the specific role of local ultrafast motions remains widely unknown. However, their relevance has recently been pointed out in connection with electronic transfer processes, such as oxidative DNA damage.[9]

Experimental methodologies for studying ultrafast conformational changes or reorganization dynamics in DNA are generally based upon spectroscopic monitoring of specific chromophores attached to DNA. Berg et al. have recently presented an approach[7] which is analogous to conventional time-resolved spectroscopy applied to studying solvation dynamics in polar solvents: Upon photoexcitation, a charge transfer (CT) chromophore undergoes an instantaneous change in its permanent electric dipole moment. The polarizibility of the surrounding medium is responding to the change of the dipole moment, and the reorientation of the environment can be followed by measuring the fluorescence Stokes shift as a function of time. From these experiments, one can derive an effective polarity of DNA as well as characteristic time scales for reorganization dynamics.[7]

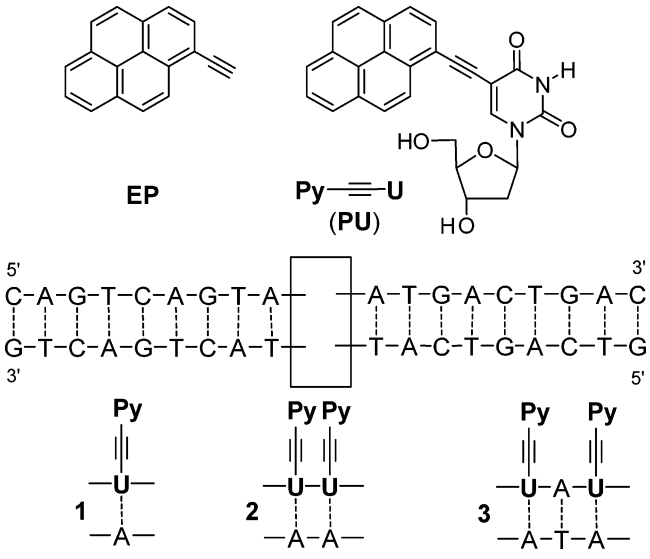
In this paper, we present an alternative approach to measuring ultrafast structural dynamics in DNA. Instead of using a *single* polar CT chromophore which is inserted into the base stack, we utilize a *pair* of interacting chromophores covalently attached to a specific DNA base but located in the major groove. This approach reduces structural perturbations in the base stack which might affect the local dynamics. When the DNA structure and conformation allow these chromophores to get in close contact, they can form *excimers* (excited dimers) with characteristic spectral properties.[3,10] Pyrene (Py)-labeled oligonucleotides have been employed to probe conformational changes in both RNA and DNA[11,12] and to investigate photoinduced charge transfer between bases in DNA.[13−16] Recently, pairs of ethynylpyrene-labeled bases have been utilized to monitor salt-induced B−Z DNA transitions by steady-state spectroscopy.[16] Previous studies on pyrene directly linked to nucleotides in the base stack revealed evidence for ultrafast electron transfer (with uracil (dU) and cytidine (dC)) and hole transfer with guanine.[14] However, the free energies for these processes have been estimated to be only around −0.1 to −0.3 eV.[15] Although an acetylene bridge is a strong mediator for electronic coupling between chromophores,[17] the additional separation between Py and dU should inhibit a complete electron transfer. The three modified DNA duplexes and reference molecules have been synthesized and investigated (Scheme 1).

The ethynylpyrene (**EP**) chromophore has been covalently attached to the 5-position of uridines, first synthesized by Korshun et al.[18] Hence, the chromophore replaces the methyl group of naturally occurring thymidines and is expected to be located in the major groove.[19] This assumption is based on similar modifications of other research groups which indicated that conformationally rigid alkynyl substitutions (when they are placed in the 5-position of pyrimidine bases) have only little influence on the stability of the resulting modified DNA duplexes and do not perturb the Watson−Crick base pairing ability.[20] Consequently, this modification strategy is now routinely applied for the attachment of fluorophores by our group[21,22] and others.[12,16,17,23] Most recently, it was shown that 5-alkylnyl substituents of uridine are able to affect the DNA duplex stabilities but more importantly, even the hydrophobic pyrenyl butyamidopropyne does not prevent the sequence-specific base pairing.[24] It is important to point out that using this molecular design, the chromophore does not replace a DNA base or a base pair which would result in an artificially constructed local environment of the chromophore inside the DNA. In our system, the **EP** group merely extends the

* To whom correspondence should be addressed. E-mail: fiebig@bc.edu.
† Boston College.
‡ Technical University of Munich.

10.1021/jp052108c CCC: $30.25
Published on Web 09/22/2005

SCHEME 1: Structures of EP, Free PU, and Three Py-≡-dU-modified DNA Duplexes 1−3



conjugated planar system of the uridine and thus equips it with optically traceable properties.

To calibrate our method, we also synthesized the free Py-≡-dU (**PU**) and investigated its spectroscopic properties in two organic solvents: methanol (MeOH, protic) and acetonitrile (MeCN, aprotic). We applied our well-established femtosecond broadband pump−probe method[25] which enables us to probe simultaneously over a large spectral range from the UV to the near-IR. A key advantage of broadband pump−probe over emission spectroscopy is the simultaneous detection of multiple electronic transitions. In the case presented here, this feature allows us to identify two independent pathways for electronic coupling between the pyrenyl chromophores.

2. Experimental Section

Femtosecond Broadband Pump−Probe Spectroscopy. A detailed description of our experimental setup has been given elsewhere.[25] Briefly, the pump wavelength was set to 364 nm for all samples. The changes in optical density were probed by a femtosecond white-light continuum (WLC) generated by tight focusing of a small fraction of the output of a commercial Ti:Sa based pump laser (CPA-2001/CPA-2010, Clark-MXR) into a 3 mm calcium fluoride (CaF_2) plate. The obtained WLC provides a usable probe source between 300 and 1200 nm. The WLC was split into two beams (probe and reference) and focused into the sample using reflective optics. Both probe and reference beams were spectrally dispersed after passing through the sample and simultaneously detected on a CCD sensor. The pump pulse (364 nm, 1 kHz, 150 and 20 nJ, respectively) was generated by frequency doubling of the compressed output of a home-built NOPA system (728 nm, 12 μJ, 40 fs). To compensate for group velocity dispersion in the UV pulse, we used an additional prism compressor. The overall time resolution of the setup is determined by the cross-correlation function between pump and probe pulses which is typically 120−150 fs (fwhm, assuming a Gaussian line shape). A spectral resolution of 7−10 nm was obtained. All measurements were performed with magic angle (54.7°) setting for the polarization of the pump with respect to the polarization of the probe pulse. A sample cell with 1.25 mm fused silica windows and an optical path of 1 mm was used for all measurements. No indications for degradation of the samples were found. The sample concentration was <1 mM. Typical optical densities of 0.8−1.0 (at the excitation wavelength) were obtained in a 1 mm cell.

Materials and Methods. Solvents were dried according to standard procedures. All reactions were carried out under argon. Chemicals were purchased from Sigma-Aldrich or Lancaster and used without further purification. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) was performed in the analytical facility of the department on a Bruker Biflex III spectrometer using 3-hydroxypicolinic acid in aqueous ammonium citrate as the matrix. C18-RP analytical and semipreparative HPLC columns (300 Å) were purchased from Supelco. All steady-state spectroscopic measurements were performed in quartz glass cuvettes (1 cm) from Starna Cells. Absorption spectra were recorded on Varian Cary 50 and Cary 100 spectrometers. The temperature for the melting curves was controlled by a Cary temperature control unit and was increased from 10 to 80 °C at a heating rate of 0.5 °C/min. The fluorescence spectra were recorded on a Fluoromax-3 fluorimeter (Jobin-Yvon). All emission spectra were recorded with a band-pass of 2 nm for both excitation and emission and are intensity corrected.

Preparation of 5-(1-Ethynylpyrenyl)-2′-deoxyuridine (PU). The synthesis was performed according to published procedures.[18]

Preparation of Oligonucleotides and DNA Duplexes. The unmodified oligonucleotides were prepared on an Expedite 8909 DNA synthesizer from Applied Biosystems via standard phosphoramidite chemistry. Phosphoramidites and CPGs (1 μmol) were purchased from Glen Research or ABI; all other synthesizer chemicals were from ABI. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with concentrated NH_4OH at 60 °C for 10 h. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A = NH_4OAc buffer (50 mM), pH = 6.5; B = MeCN; gradient = 0−15% B over 45 min. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm.[26]

Preparation and Characterization of Py-≡-dU-Modified Oligonucleotides.[27] The solid-phase synthesis sequence was stopped after the incorporation of 5′-DMT-3′-cyanoethyl-*N*,*N*′-diisopropyl phosphoramidite-2′-deoxy-5-iodoridine (Glen Research) without deprotecting the 5′-hydroxyl or cleaving the oligonucleotide from the resin. In the case of the double-labeled oligonucleotides, the synthesis was stopped after incorporation of both 5-iodo-2′-deoxyuridine moieties. The column was subsequently removed from the synthesizer and dried in vacuo. Then, the column was attached to a syringe and the reaction solution consisting of ethynylpyrene (60 μmol), $Pd(Ph_3P)_4$ (30 μmol), and CuI (30 μmol) in dry DMF/Et_3N (3.5:1.5) (0.5 mL) was injected into the column and into another syringe, attached to the other end of the column. The reaction solution was moved back and forth between the two syringes through the column several times to ensure even distribution of the reaction solution. After a coupling time of 3 h at room temperature, the reaction solution was discarded and the column was washed with DMF/Et_3N (9:1) (10 mL) and dry MeCN (40 mL), dried in vacuo, and reinstalled on the synthesizer. Solid-phase synthesis was resumed, and additional DNA bases were added. The deprotection, cleavage from resin, and purification by HPLC were performed as described above, but the gradient for HPLC purification was run from 0 to 30% MeCN over 45 min. The concentrations of the oligodeoxynucleotides were determined following the absorption at 260 nm as described previously.[22,27]

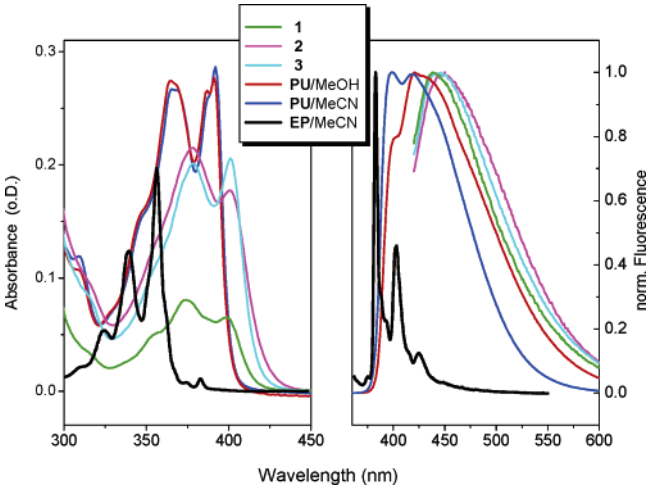


Figure 1. Steady-state absorption (left) and fluorescence (right) spectra of the duplexes **1**−**3**, **PU**, and **EP** in organic solvents (MeOH and MeCN).

Masses of the DNA strands **1**(ss), **2**(ss), and **3**(ss) were determined by MALDI-TOF mass spectrometry to confirm the correct base sequence and the successful incorporation of the Py-≡-dU group (**1**(ss), mass calcd 6022, mass exp 6027; **2**(ss), mass calcd 6538, mass calcd 6532; **3**(ss), mass calcd 6851, mass exp 6843). Duplexes with 1.2 equiv of the unmodified complementary strand were formed by heating to 80 °C (10 min), followed by slow cooling. Melting temperatures (T_m) were measured at 260 nm with **1**−**3** (1.25 μM) in Na−P_i buffer solution (10 mM) with NaCl (250 mM) and pH 7.0 (**1**, T_m 66 °C; **2**, T_m 62 °C; **3**, T_m 59 °C).

3. Results

Steady-State Absorption and Fluorescence. Figure 1 shows the absorption and fluorescence spectra of all three DNA duplexes (**1**−**3**) together with those of **PU** (in MeOH and MeCN) and **EP** (in MeCN). The absorption spectra of Py-≡-dU (both in DNA and in organic solvents) are substantially different from that of **EP**. The apparent red shift of the long-wavelength absorption band in **EP** is caused by a strong enhancement of the $S_0 \rightarrow S_1$ transition in Py-≡-dU due to intramolecular electronic coupling between Py and dU. The absorption spectra clearly indicate that the electronic structure of Py-≡-dU is delocalized. Hence, even though the pyrene moiety is located in the major groove, the optical excitation will spread instantly over the entire molecule and thus into the base stack. In turn, the π-stacking interactions affect the pyrenyl absorption bands as indicated by the pronounced red shift (~10 nm) of the duplexes **1**−**3**, with respect to the maxima of **PU** in MeOH and MeCN.

Because of the strong intramolecular coupling, the typical pyrenyl-type fluorescence (as observed for **EP**) is absent in Py-≡-dU. Hence, one cannot expect to find the characteristic red shifted emission, typically observed in pyrene excimers. Instead, the fluorescence spectra of the "dimer" duplexes **2** and **3** show only a moderate red shift in their fluorescence spectra.[28]

Femtosecond Broadband Pump−Probe Spectroscopy. Figure 2 displays the temporal evolution of the pump−probe spectra of **1** and **PU** within 1 ns after optical excitation. The spectra are dominated by a broad, asymmetric absorption band with a maximum around 700 nm which must be attributed to (Py-≡-dU)* absorption. Note that the **PU** in MeOH and Py-≡-dU inserted in the base stack (**1**) exhibits very similar spectral dynamics. The onset of relaxation in the excited state is

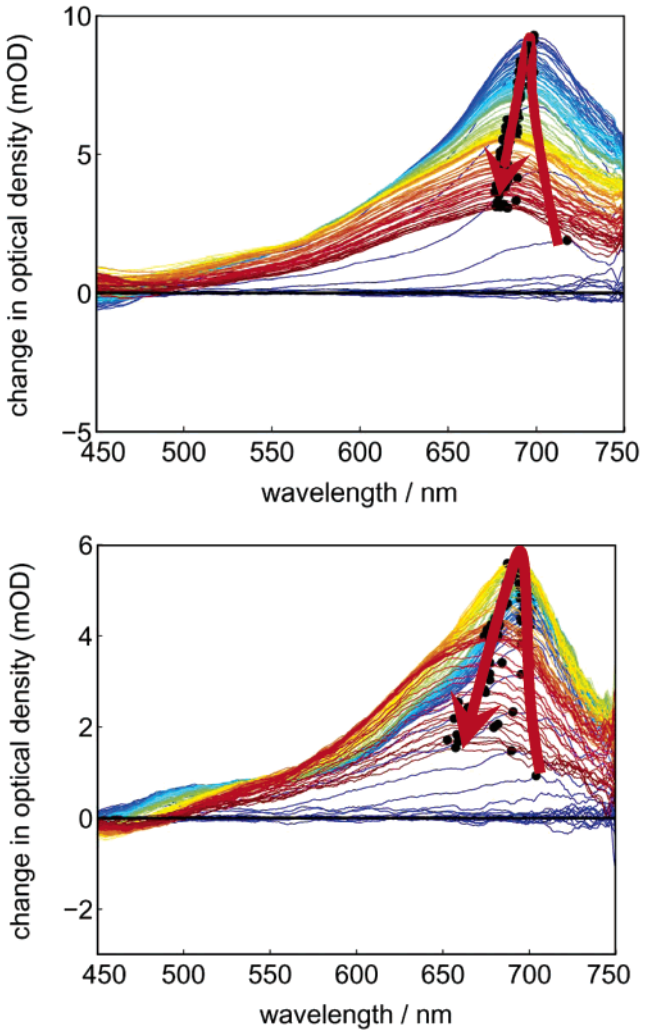


Figure 2. Temporal evolution of the pump−probe spectra of **1** (top) and **PU** in MeOH (bottom) in the time range between −1 ps and 1 ns after excitation at 364 nm (various step sizes). Early spectra are shown in blue/green and late spectra are shown in orange/red colors. The red arrows trace the time-dependent spectral maxima (see text for details).

manifested by a spectral blue shift of the absorption band. The spectral shifting dynamics are continuous, similar to those observed in time-resolved emission studies of CT states in polar solvents.[29] Since analogous pump−probe measurements on **PU** in MeCN (data not shown) do not show a blue shift, it must be assumed that the latter originates from a solvation process involving rearrangements in the hydrogen bond network. A similar observation has been made for Py−dU, where the chromophores are directly linked through a covalent bond.[30]

Figure 3 shows the time-resolved pump−probe spectra of the Py-≡-dU dimers (**2** and **3**) in DNA. When these spectra are compared with those from the single Py-≡-dU chromophore (Figure 2), three important differences can be observed: First, an additional absorption band around 500 nm rises within 300 fs.[31] Second, the shifting of the spectral maximum at ~700 nm to shorter wavelengths is more pronounced. Third, the spectra of **2** and **3** are broader (throughout the entire time range) than the ones of **1**.

A quantitative comparison of the spectral shifting dynamics in **1**−**3** is shown in Figure 4 where the spectral position of the maxima of the (Py-≡-dU)* absorption band are plotted as a function of time. All peak shift functions have been fitted using biexponentials (see Table 1). In **1**, the (Py-≡-dU)* absorption band undergoes a rapid shift with a time constant of 4.6 ps

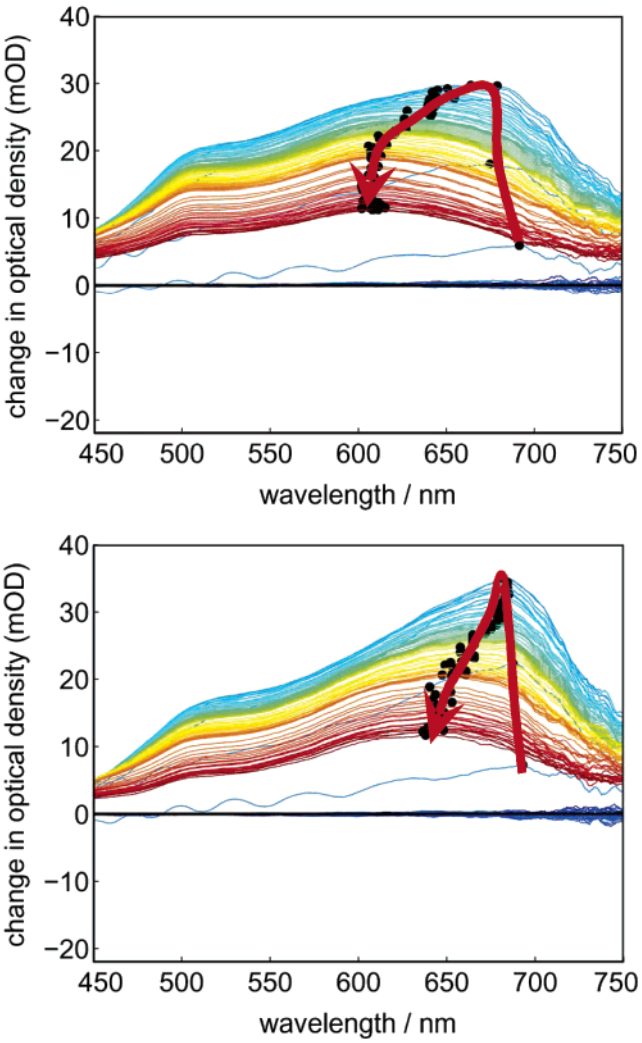


Figure 3. Temporal evolution of the pump−probe spectra of **2** (top) and **3** (bottom) in the time range between −1 ps and 1 ns after excitation at 364 nm (various step sizes). Early spectra are shown in blue/green and late spectra are shown in orange/red colors. The red arrows trace the time-dependent spectral maxima (see text for details).

TABLE 1: Parameters of the Peak Shift Functions for Duplexes 1−3 Obtained from a Least-Square Fit Using a Biexponential Decay Function

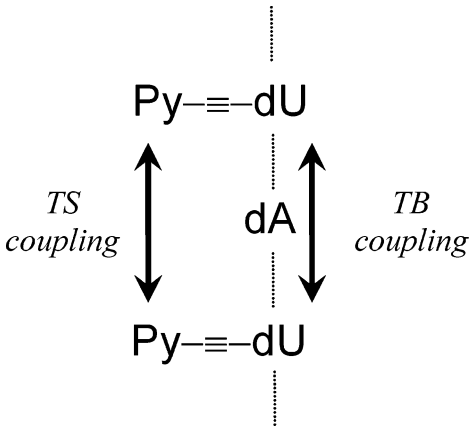
	$\Delta\tilde{\nu}$ (cm^{-1})	τ_1 (ps)	τ_2 (ps)	A_1 (%)	A_2 (%)
1	440	4.6	220	69	31
2	1300	1.9	7.6	85	15
3	1000	16.4	∼2000	47	53

(70%), followed by a slower shift of 220 ps (30%). However, the total magnitude of the shift is only 440 cm^{-1}. In contrast, in **2**, the spectral shift is 1300 cm^{-1} with a time constant of ∼2 ps for 85%. In **3**, one observes almost the same magnitude of spectral shifting (1000 cm^{-1}), however, exhibited on a much longer time scale; approximately 50% of the shift occurs with a 16 ps time constant and 50% on a significantly longer time scale of 1.9 ns.

4. Discussion

From the temporal evolution of the pump−probe spectra of **1**−**3**, a detailed picture of the spectral and dynamical characteristics of chromophore interactions through DNA bases emerges. The results shown above clearly indicate the existence of two pathways for excitation energy transfer, mediated by through-space and through-base stack interactions.

SCHEME 2: Qualitative Schematics of the Two Electronic Coupling Pathways for Excitation Energy Transfer in 3 (and 2): Through-Space and Through-Base Stack Coupling[a]



[a] It should be noted that there is a substantial amount electronic energy and charge redistribution in the excited state of **Py-≡-dU**, which accounts for the Stokes' shifted emission spectra (compared to pyrene, see Figure 1). In addition, two **Py-≡-dU** moieties can interact with each other in the same fashion as aromatic hydrocarbons, leading to mainly resonance stabilized excimer-type excited states: (Py=dU)* ⋯ Py=dU ↔ Py=dU ⋯ (Py=dU)*.

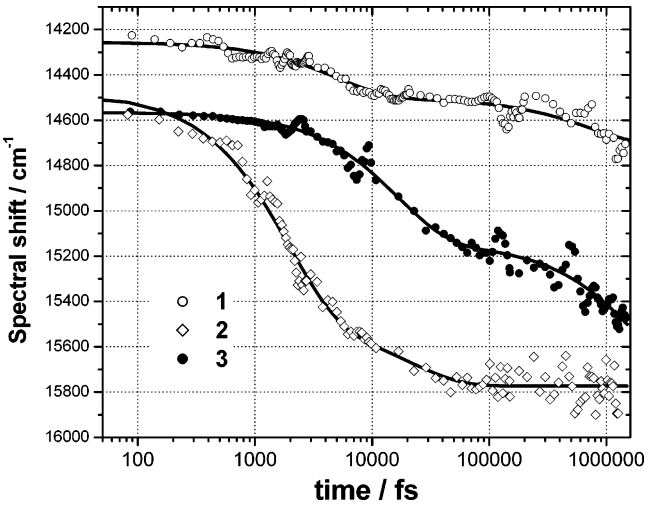


Figure 4. Spectral positions of the (Py=dU)* absorption maxima plotted as a function of time for **1**−**3**.

Through-Space (TS) Interaction. The drastic spectral shifts in the broadband excited-state absorption spectra of **2** and **3** (compared to **1**) clearly certify dynamic interactions between the two chromophores. The differences in the observed magnitudes of the spectral shifts ($\Delta\tilde{\nu}$) (Table 1) suggest that stabilizing chromophore interactions between the two pyrene chromophores are present. It therefore seems reasonable to assume that an excimer-type state is being formed in **2** and **3**. The (Py=dU)* absorption band exhibits a dynamical solvatochromic shift, similar to those observed in time-resolved fluorescence spectroscopy. However, in fluorescence spectroscopy, excited-state processes (e.g., charge transfer) lead to shifts toward longer wavelengths while the opposite behavior (i.e., shifting toward shorter wavelengths) is observed in excited-state absorption. In the case of **3**, where the pyrene moieties are separated by one intervening AT base pair, forming a stabilized excimer structure requires substantial conformational reorganization (see Figure 5) involving at least three base pairs and takes place on the nanosecond time scale. The fact that $\Delta\tilde{\nu}$

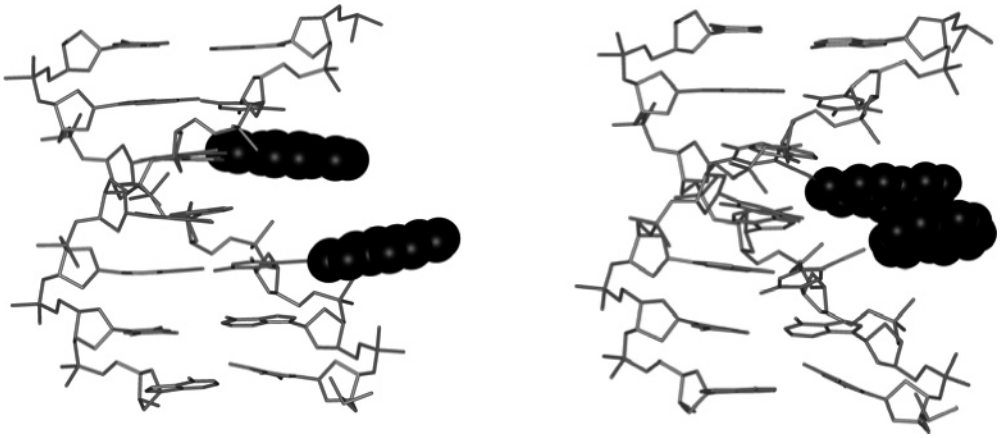


Figure 5. Molecular modeling (Amber force field) of duplex **3** in two different conformations: left, standard B-DNA structure (based on nucleic acid builder) and right, suggested excimer structure with maximum π-overlap between the two attached pyrene moieties.

is similar for **3** and **2** suggests that excimers of similar structure are formed in both duplexes. However, the time scale on which the spectral shift occurs is different. In **3**, 50% of the shift takes place with a time constant of 16 ps. The finalization of the shift, however, takes place on a much longer time scale ($\sim$2 ns). In 16 ps, only small structural adjustments of the two chromophores are possible. Since the base−acetylene−pyrene axis is fairly rigid, it is likely that bending motions and partial rotation would have to originate from the base pair. A tightly bound excimer structure could be achieved by additional local reorganization of the entire duplex. Those "large-scale" reorientations are likely to take place on the nanosecond time scale.

Because of the intrinsic asymmetry in the structure of Py-≡-dU, there are at least two possible relative orientations of the pyrene moieties to be considered. Since both orientations will be present in the ground state, it is expected to have spectral inhomogeneities in the excited-state absorption spectrum and thus broader bands in the case of **2** and **3** (relative to **1**). With one base pair separating the two chromophores, the distance between the pyrene moieties increases to $\sim$7 Å. Moreover, the additional pitch of 36° leads to a vanishing π-overlap in the equilibrium structure (see Figure 5, left).

Through-Base Stack (TB) Interaction. Since the excitation is delocalized over the Py≡dU chromophore, the optical excitation leads to instant electronic excitation in the base stack. The appearance of the excited-state absorption band at 500 nm in both **2** and **3** within 300 fs after optical pumping indicates efficient excitation energy transfer mediated through the intervening AT base pair. Note that the magnitude of the electronic coupling which gives rise to a new absorption band and the ultrafast rate of the process suggests that the energy transfer is mediated through direct π-orbital overlap (as opposed to dipolar Coulombic coupling!).

Finally, it is important to note that both electronic transitions in dimer systems **2** and **3** (i.e., the 500 and 700 nm band) must originate for the same excited state since the decay times of both absorption bands are identical within the experimental error. Although TB mediated transfer is accompanied by a very small structural reorganization, in contrast to pyrenyl excimer formation, the electronic nature of both product states must be very similar. Hence, we are probing simultaneously two different regions of the multidimensional lowest-energy potential energy surface of the system.

5. Conclusion

We have applied femtosecond broadband pump−probe spectroscopy to monitor local structural dynamics in DNA utilizing two chromophores which were covalently (rigidly) attached to DNA bases but spatially extended into the DNA groove. Our results indicate the presence of two electronic coupling pathways. First, a very efficient through-base stack pathway that leads to a new transient absorption band in the dimer systems (**2** and **3**). The bands rise on the time scale of 300 fs, even in the case when the two chromophores are separated by one AT base pair. Hence, it must be concluded that excitation energy can migrate over distances of $\sim$7 Å in $\sim$300 fs. Since the ground-state structure should not permit direct spatial π-orbital overlap between the chromophores, it is likely that the interaction is facilitated through indirect coupling involving excited states of the intervening AT base pair.

The second interaction pathway, which involves direct orbital overlap, has been identified by a pronounced dynamic spectral blue shift. From the analysis of the time-dependent shift, we extracted the time scale for local structural motions in DNA. As expected, if the chromophores are in direct contact, then only minor structural changes are needed to form a stable excimer structure. Hence, the shifting dynamics are completed after 10 ps. In the case where the chromophores were separated by one AT base pair, the overall shifting dynamics exhibit a long time component of $\sim$2 ns. We assign this time constant to local structural motions in DNA involving the reorientation of at least three base pairs.

The results of our study are important in two ways. First, they demonstrate the ability of DNA bases to mediate ultrafast and efficient excitation energy transfer. To our knowledge, this has never been demonstrated before. The potential biological impact of this finding might be significant for a comprehensive understanding of sun-light-induced DNA damage. Second, the observed local flexibility of DNA must be considered when dynamical models for DNA−drug interactions are developed.

Acknowledgment. The work was supported by Boston College and by the Deutsche Forschungsgemeinschaft (Wa-1386/7-3). We are thankful to Qiang Wang for experimental assistance. We would also like to thank the reviewers for valuable comments.

References and Notes

(1) Alam, T. M.; Drobny, G. P. *Chem. Rev.* **1991**, *91*, 1545. Perkins, T. T.; Smith, D. E.; Chu, S. *Science* **1994**, *264*, 819. Gmeiner, W. H. *Curr. Med. Chem.* **1998**, *5*, 115. Naimushin, A. N.; Fujimoto, B. S.; Schurr, J. M. *Biophys. J.* **2000**, *78*, 1498. Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253. Smith, B. L. *Prog. Biophys. Mol. Biol.* **2000**, *74*, 93. O'Neill, M. A.; Barton, J. K. *J. Am. Chem. Soc.* **2002**, *124*, 13053. Bansal, M. *Curr. Sci.* **2003**, *85*, 1556.

(2) Millar, D. P. *Curr. Opin. Struct. Biol.* **1996**, *6*, 322.
(3) Balakin, K. V.; Korshun, V. A.; Mikhalev, II.; Maleev, G. V.; Malakhov, A. D.; Prokhorenko, I. A.; Berlin, Y. A. *Biosens. Bioelectron.* **1998**, *13*, 771. Paris, P. L.; Langenhan, J. M.; Kool, E. T. *Nucleic Acids Res.* **1998**, *26*, 3789.
(4) Okonogi, T.; Reese, A. W.; Alley, S. C.; Hopkins, P. B.; Robinson, B. H. *Biophys. J.* **1999**, *77*, 3256.
(5) Shipway, A. N.; Willner, I. *Chem. Commun.* **2001**, 2035. Sponer, J.; Leszczynski, J.; Hobza, P. *Biopolymers* **2001**, *61*, 3. Cui, D. X.; Gao, H. J. *Biotechnol. Prog.* **2003**, *19*, 683. Robertson, N.; McGowan, C. A. *Chem. Soc. Rev.* **2003**, *32*, 96. Endres, R. G.; Cox, D. L.; Singh, R. R. P. *Rev. Mod. Phys.* **2004**, *76*, 195. Gao, H. J.; Kong, Y. *Annu. Rev. Mater. Res.* **2004**, *34*, 123. Iguchi, K. *Int. J. Mod. Phys. B* **2004**, *18*, 1845.
(6) Cheatham, T. E. *Curr. Opin. Struct. Biol.* **2004**, *14*, 360.
(7) Brauns, E. B.; Madaras, M. L.; Coleman, R. S.; Murphy, C. J.; Berg, M. A. *J. Am. Chem. Soc.* **1999**, *121*, 11644. Somoza, M. M.; Andreatta, D.; Murphy, C. J.; Coleman, R. S.; Berg, M. A. *Nucleic Acids Res.* **2004**, *32*, 2494.
(8) Brauns, E. B.; Madaras, M. L.; Coleman, R. S.; Murphy, C. J.; Berg, M. A. *Phys. Rev. Lett.* **2002**, *88*, 158101.
(9) Berlin, Y. A.; Burin, A. L.; Siebbeles, L. D. A.; Ratner, M. A. *J. Phys. Chem. A* **2001**, *105*, 5666. O'Neill, M. A.; Becker, H. C.; Wan, C. Z.; Barton, J. K.; Zewail, A. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 5896. Troisi, A.; Nitzan, A.; Ratner, M. A. *J. Chem. Phys.* **2003**, *119*, 5782. O'Neill, M. A.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 11471. Kaden, P.; Mayer-Enthart, E.; Trifonov, A.; Fiebig, T.; Wagenknecht, H.-A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1636.
(10) Dioubankova, M. N.; Malakhov, A. D.; Stetsenko, D. A.; Gait, M. J.; Volynsky, P. E.; Efremov, R. G.; Korshun, V. A. *ChemBioChem* **2003**, *4*, 841. Yamana, K.; Takei, M.; Nakano, H. *Tetrahedron Lett.* **1997**, *38*, 6051. Christensen, U. B.; Pedersen, E. B. *Helv. Chim. Acta* **2003**, *86*, 2090. Lewis, F. D.; Zhang, Y.; Letsinger, R. L. *J. Am. Chem. Soc.* **1997**, *119*, 5451. Hrdlicka, P. J.; Babu, B. R.; Sorensen, M. D.; Wengel, J. *Chem. Commun.* **2004**, 1478.
(11) Korshun, V. A.; Pestov, N. B.; Birikh, K. R.; Berlin, Y. A. *Bioconjugate Chem.* **1992**, *3*, 559. Kool, E. T. *Chem. Rev.* **1997**, *97*, 1473. Kool, E. T. *Acc. Chem. Res.* **2002**, *35*, 936. Beuck, C.; Singh, I.; Bhattacharya, A.; Heckler, W.; Parmar, V. S.; Seitz, O.; Weinhold, E. *Angew. Chem., Int. Ed.* **2003**, *42*, 3958. Prokhorenko, I. A.; Korshun, V. A.; Petrov, A. A.; Gontarev, S. V.; Berlin, Y. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2081. Korshun, V. A.; Balakin, K. V.; Proskurina, T. S.; Mikhalev, II; Malakhov, A. D.; Berlin, Y. A. *Nucleosides Nucleotides* **1999**, *18*, 2661. Mann, J. S.; Shibata, Y.; Meehan, T. *Bioconjugate Chem.* **1992**, *3*, 554. Kierzek, R.; Li, Y.; Turner, D. H.; Bevilacqua, P. C. *J. Am. Chem. Soc.* **1993**, *115*, 4985. Yamana, K.; Gokota, T.; Ozaki, H.; Nakano, H.; Sangen, O.; Shimidzu, T. *Nucleosides Nucleotides* **1992**, *11*, 383.
(12) Okamoto, A.; Kanatani, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820.
(13) Manoharan, M.; Tivel, K. L.; Zhao, M.; Nafisi, K.; Netzel, T. L. *J. Phys. Chem.* **1995**, *99*, 17461. Netzel, T. L.; Zhao, M.; Nafisi, K.; Headrick, J.; Sigman, M. S.; Eaton, B. E. *J. Am. Chem. Soc.* **1995**, *117*, 9119. Netzel, T. L.; Nafisi, K.; Headrick, J.; Eaton, B. E. *J. Phys. Chem.* **1995**, *99*, 17948. Amann, N.; Pandurski, E.; Fiebig, T.; Wagenknecht, H.-A. *Angew. Chem., Int. Ed.* **2002**, *41*, 2978. Amann, N.; Pandurski, E.; Fiebig, T.; Wagenknecht, H.-A. *Chem. −Eur. J.* **2002**, *8*, 4877. Zahavy, E.; Fox, M. A. *J. Phys. Chem. B* **1999**, *103*, 9321.
(14) Huber, R.; Fiebig, T.; Wagenknecht, H. A. *Chem. Commun.* **2003**, 1878.
(15) Raytchev, M.; Mayer, E.; Amann, N.; Wagenknecht, H. A.; Fiebig, T. *ChemPhysChem* **2004**, *5*, 706.
(16) Okamoto, A.; Ochi, Y.; Saito, I. *Chem. Commun.* **2005**, 1128.
(17) Thoresen, L. H.; Jiao, G. S.; Haaland, W. C.; Metzker, M. L.; Burgess, K. *Chem. −Eur. J.* **2003**, *9*, 4603. Hurley, D. J.; Tor, Y. *J. Am. Chem. Soc.* **2002**, *124*, 3749. Jiao, G. S.; Kim, T. G.; Topp, M. R.; Burgess, K. *Org. Lett.* **2004**, *6*, 1701. Hwang, G. T.; Seo, Y. J.; Kim, B. H. *J. Am. Chem. Soc.* **2004**, *126*, 6528.
(18) Korshun, V. A.; Prokhorenko, I. A.; Gontarev, S. V.; Skorobogatyi, M. V.; Balakin, K. V.; Manasova, E. V.; Malakhov, A. D.; Berlin, Y. A. *Nucleosides Nucleotides* **1997**, *16*, 1461. Malakhov, A. D.; Malakhova, E. V.; Kuznitsova, S. V.; Grechishnikova, I. V.; Prokhorenko, I. A.; Skorobogatyi, M. V.; Korshun, V. A.; Berlin, Y. A. *Bioorg. Khim.* **2000**, *26*, 39.
(19) Barnes, T. W.; Turner, D. H. *J. Am. Chem. Soc.* **2001**, *123*, 4107. Znosko, B. M.; Barnes, T. W.; Krugh, T. R.; Turner, D. H. *J. Am. Chem. Soc.* **2003**, *125*, 6090.
(20) Freier, S. M.; Altmann, K. H. *Nucleic Acids Res.* **1997**, *25*, 4429. Demesmaeker, A.; Haner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, *28*, 366.
(21) Wagner, C.; Rist, M.; Mayer-Enthart, E.; Wagenknecht, H. A. *Org. Biomol. Chem.* **2005**, *3*, 2062.
(22) Mayer, E.; Valis, L.; Wagner, C.; Rist, M.; Amann, N.; Wagenknecht, H. A. *ChemBioChem* **2004**, *5*, 865.
(23) Hwang, G. T.; Seo, Y. J.; Kim, S. J.; Kim, B. H. *Tetrahedron Lett.* **2004**, *45*, 3543.
(24) Kottysch, T.; Ahlborn, C.; Brotzel, F.; Richert, C. *Chem. −Eur. J.* **2004**, *10*, 4017.
(25) Raytchev, M.; Pandurski, E.; Buchvarov, I.; Modrakowski, C.; Fiebig, T. *J. Phys. Chem. A* **2003**, *107*, 4592.
(26) Puglisi, J. D.; Tinoco, I. *Methods Enzymol.* **1989**, *180*, 304.
(27) Rist, M.; Amann, N.; Wagenknecht, H. A. *Eur. J. Org. Chem.* **2003**, 2498.
(28) It should be noted that there is a substantial amount electronic energy and charge redistribution in the excited state of **Py═dU**, which accounts for the Stokes' shifted emission spectra (compared to pyrene, see Figure 1). In addition, two **Py═dU** moieties can interact with each other in the same fashion as aromatic hydrocarbons, leading to mainly resonance stabilized excimer-type excited states: (Py═dU)* ⋯ Py═dU ↔ Py═dU ⋯ (Py═dU)*.
(29) Barbara, P.; Jarzeba, W. Ultrafast Photochemical Intramolecular Charge Transfer and Excited-State Solvation. In *Advances in Photochemistry*; Volman, D. H., G. S. H., Gollnick, K., Eds.; Ed.; Wiley: Weinheim, Germany, 1990; Vol. 15, p 1. Maroncelli, M. *J. Mol. Liq.* **1993**, *57*, 1.
(30) Trifonov, A.; Buchvarov, I.; Wagenknecht, H.-A.; Fiebig, T. *Chem. Phys. Lett.* **2005**, *409*, 277.
(31) In principle, the pump−probe signal at 500 nm (in **2** and **3)** could result from a spectral superposition of (positive) excited-state absorption and (negative) stimulated emission. This possibility was ruled out for the following reason: A positive band (as observed in **2** and **3)** would result from a smaller contribution of stimulated emission in **2** and **3** relative to **1**. However, the relative fluorescence quantum yields indicate the exact opposite behavior, for example, the quantum yield of **3** is approximately twice as large as the one for **1**.