DNA

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Ultraviolet Absorption Induces Hydrogen-Atom Transfer in G·C Watson–Crick DNA Base Pairs in Solution

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Abstract: *Ultrafast deactivation pathways bestow photostability on nucleobases and hence preserve the structural integrity of DNA following absorption of ultraviolet (UV) radiation. One controversial recovery mechanism proposed to account for this photostability involves electron-driven proton transfer (EDPT) in Watson–Crick base pairs. The first direct observation is reported of the EDPT process after UV excitation of individual guanine–cytosine (G·C) Watson–Crick base pairs by ultrafast time-resolved UV/visible and mid-infrared spectroscopy. The formation of an intermediate biradical species (G[−H]·C[+H]) with a lifetime of 2.9 ps was tracked. The majority of these biradicals return to the original G·C Watson–Crick pairs, but up to 10 % of the initially excited molecules instead form a stable photoproduct G*·C* that has undergone double hydrogen-atom transfer. The observation of these sequential EDPT mechanisms across intermolecular hydrogen bonds confirms an important and long debated pathway for the deactivation of photoexcited base pairs, with possible implications for the UV photochemistry of DNA.*

For over fifty years, the role of interstrand proton or hydrogen-atom transfer in double-helix DNA has been debated as a possible precursor for mutagenesis and carcinogenesis.[1] However, recent theoretical studies postulated that ultrafast interstrand electron-driven proton transfer (EDPT) instead contributes to the prevention of mutagenic photolesions in DNA excited by absorption of solar ultraviolet (UV) radiation.[2] Rapid relaxation processes such as the proposed EDPT pathway render DNA intrinsically photostable[3] and reduce the need for enzyme-driven repair[4] of photodamage. Despite extensive prior study of DNA photophysics, the question of whether UV-induced EDPT is active in Watson–Crick (WC) base pairs remains contentious, and contradictory experimental results have been published.[2a,5] Recent reports suggest that in duplex DNA, both intrastrand interactions between "vertically" stacked nucleobases attached to the same sugar–phosphate backbone and interstrand interactions between "horizontally" WC-paired bases might contribute to the photochemistry of double-stranded DNA.[6] A hybrid of the two processes may also occur; Zhang et al. invoked interstrand proton transfer after intrastrand electron transfer,[5d] but considered pure interstrand EDPT to be unlikely on the basis of QM/MM calculations on duplex DNA.[7] The vertical π-stacking interactions can also promote the photoinduced formation of long-lived excimers,[6] but whether these excimers initiate or suppress proton-transfer reactions is unresolved.

Herein, we describe the use of ultrafast time-resolved optical spectroscopy, in both the UV/visible and mid-infrared (IR) spectral regions, to track the decay dynamics of an ensemble of individual, UV-excited G·C WC base pairs (**1**) in solution. These results, summarized in Figure 1, show direct evidence for the involvement of EDPT in the deactivation dynamics of a G·C WC pair. Observation in a solution of G·C dimers excludes any possible participation of excimer states induced by π-stacking. After UV excitation of G·C in chloroform, a single hydrogen atom is transferred within 40 fs with a quantum yield of $\Phi_{\text{biradical}} \geq 0.6$, forming an intermediate biradical species (G[−H]·C[+H]), which either recovers to the original G·C WC pair or decays to generate a "stable" (within the 1.3 ns timeframe of our measurements) double-hydrogen-atom-transferred photoproduct (G*·C*). This work provides the most compelling evidence to date for the involvement of EDPT-driven relaxation in individual WC base pairs and identifies the mechanism through which this process proceeds.

Time-resolved electronic absorption spectroscopy (TEAS) and time-resolved vibrational absorption spectroscopy (TVAS) measurements of the G·C base pair were performed with equimolar solutions of silyl-protected guanosine (G) and (deoxy)cytidine (C) in chloroform (for experimental details, see the Supporting Information). In this aprotic solvent, the G and C mixtures exist predominantly in the WC conformation.[5c,8] Moreover, chloroform provides a reasonable model for the dielectric environment in the core of natural DNA.[9] At an excitation wavelength of 260 nm, about 80 % of the photons are absorbed by G (see the Supporting Information, Figure S5).[5c,8a] Excitation at the red edge of the absorption spectrum at 290 nm promotes the same

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Supporting information for this article, including summaries of the experimental details, data analysis procedures, and outcomes, is available on the WWW under http://dx.doi.org/10.1002/anie.201506940. All experimental data are archived in the University of Bristol's Research Data Storage Facility.

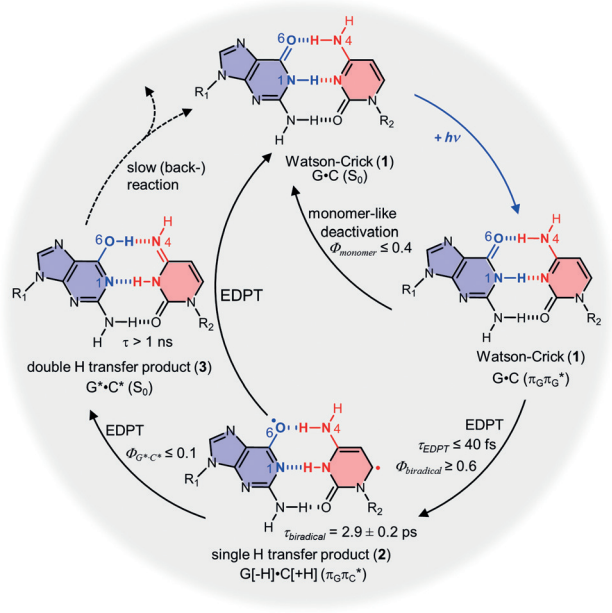

Figure 1. Deactivation mechanism of electronically excited G·C Watson–Crick base pairs (**1**). After UV excitation of the guanine moiety (blue), EDPT along the central hydrogen bond occurs within 40 fs, and the resulting G[−H]·C[+H] biradical (**2**) has a lifetime of 2.9 ps. This intermediate undergoes a second EDPT, which either returns it to the original ground-state Watson–Crick structure or leads to the G*·C* tautomer (**3**). The G*·C* tautomer is stable for >1 ns, although its eventual fate is unknown. Alternatively, the initially excited G·C molecules can undergo monomer-like deactivation pathways. The quantum yields (Φ) correspond to 260 nm excitation.

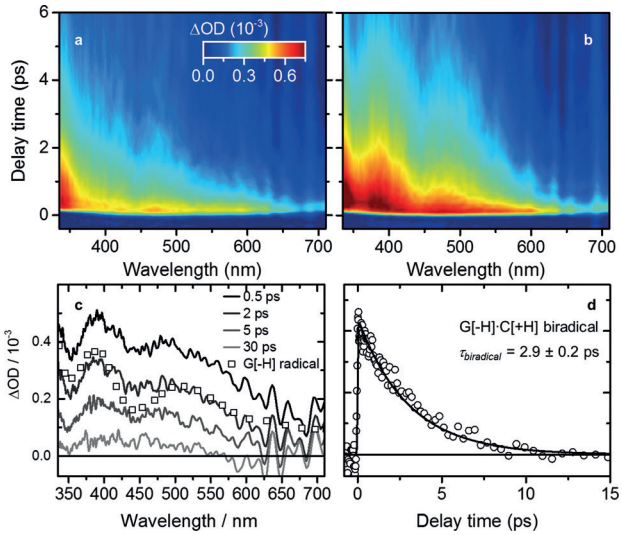

Figure 2. Transient electronic absorption spectra of the G·C Watson–Crick base pair after excitation at 260 nm. a) Superposition of the transient absorption changes of G and C solutions measured separately in $CHCl_3$. b) Experimental transient absorption changes of an equimolar mixture of G and C in $CHCl_3$. c) Transient differences between the spectra in (b) and (a) at selected delay times and normalized spectrum of the G[−H] radical.[10b] d) Time profile of the difference spectrum (○) and fit (——) at 400 nm. All spectra were smoothed by replacing each data point of the two-dimensional transient absorption changes with the average of a 3×3 neighborhood.

photochemistry, but the product bands observed in TEAS and TVAS are weaker because of the lower absorption by G at this wavelength. Excitation of C leads to monomer-like deactivation (see Section S12). Therefore, the discussion in this paper focuses on the results after 260 nm excitation.

Figures 2a and 2b show a superposition of the TEAS results for separate G and C solutions and for an equimolar mixture of G and C, respectively, all in $CHCl_3$. The transient absorption map in Figure 2b is dominated by the G·C WC base pair and exhibits pronounced structure with maxima at 390 nm and 500 nm. Figure 2c compares transient difference spectra between the signals in Figure 2a and 2b at selected delay times with the known spectrum of the G[−H] radical.[10] This radical is one of the key intermediates in the EDPT process (Figure 1), and its observation demonstrates the involvement of the EDPT pathway in the electronic deactivation of the G·C WC base pair. The partner C[+H] radical absorbs only weakly in this spectral region (see Section S11, Figure S9). The absorption spectrum of the $G^{+\cdot}$ radical cation is similar to that of G[−H], but arguments detailed in Section S16 and S17 exclude this alternative assignment. The lifetime of the EDPT intermediate is 2.9 ± 0.2 ps (Figure 2d; uncertainties are 2 standard deviations throughout), which supports calculations of a minimum on the excited-state potential energy surface (PES),[11] rather than direct deactivation to the electronic ground state (S_0). The prompt absorption rise indicates that EDPT product formation is faster than the experimental time resolution (ca. 40 fs), and hence a lower limit to the quantum yield of $\Phi_{\text{biradical}} \geq 0.6 \pm 0.1$ (see Section S8). Prior calculations[2b] identified crossing from the photoexcited $\pi_G\pi_G^*$ state to a $\pi_G\pi_C^*$ charge transfer (CT) state as the driving force for EDPT to G[−H]·C[+H]. The fast population of the CT state is in accord with ab initio molecular dynamics simulations of the G·C WC base pair.[12] The oxygen-centered radical shown in Figure 1 is expected to be the most stable biradical structure[10a] and provides a favorable starting point for a second hydrogen-atom transfer along the $N_{4,C}$–H···$O_{6,G}$ bond.

To explore the fate of the G[−H]·C[+H] biradical, we performed TVAS experiments after excitation at 260 nm. Figure 3 displays the results, together with calculated IR spectra for the G·C WC base pair (**1**) and the G*·C* tautomer (**3**) arising from double hydrogen-atom transfer (Figure 1). The transient spectra in Figure 3a show three distinct negative contributions (bleaches), which match the steady-state IR spectrum of the G·C pair (Figure 3b1) and reflect population transfer to electronic excited states. The positive features at 1680 cm^{-1}, 1630 cm^{-1}, and 1580 cm^{-1} decay with increasing delay time. As seen in other systems,[13] they can be assigned predominantly to vibrationally hot S_0 WC pairs at the $\nu = 1$ level, either of the vibrational mode responsible for the adjacent bleach feature or of a coupled mode. However, the small positive band at 1720 cm^{-1} has no corresponding bleach feature at higher wavenumbers and shows no decay after its growth within the range of our experiment (1.3 ns); hence, it was attributed to a photoproduct, which also accounts for incomplete WC pair recoveries. As described in detail in Section S6, calculations performed at the B3LYP/6-311+G** level of theory for possible photoproducts,

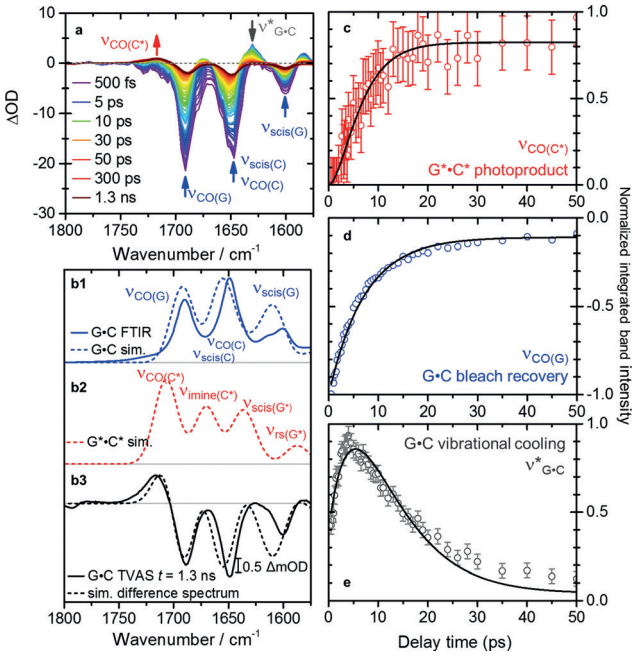

Figure 3. Transient vibrational absorption spectra, calculated vibrational spectra, and time profiles after excitation of G·C at 260 nm. a) Transient IR spectra of G·C in $CDCl_3$. b1, b2) Experimental (solid lines) and calculated IR (dashed lines) spectra for the G·C WC structure and the G*·C* tautomer. b3) Difference spectra of G*·C* and G·C (simulated assuming $\Phi = 7\%$) compared with the experimental transient spectrum at 1.3 ns. c–e) Time profiles (○) and fit results (——) to the spectral features centered at 1720 cm^{-1} (c), 1693 cm^{-1} (d), and 1630 cm^{-1} (e). Labels indicate the vibrational character of bands (see Table S2). The transient spectra were smoothed using a running average with one neighboring pixel. Calculations were performed at the B3LYP/6-311+G** level of theory.

including a number of tautomers and the G and C monomers, demonstrate that only the G*·C* tautomer matches the observed spectral characteristics. Figure 3b compares calculated and experimental spectra. The theoretical spectrum of the G·C WC pair (Figure 3b1) agrees well with the steady-state IR spectrum of G·C. The difference between the computed G·C and G*·C* spectra is shown in Figure 3b3 together with a late-time TVAS spectrum. The close resemblance indicates that a fraction of the initially excited G·C pairs indeed forms the G*·C* structure. The G*·C* quantum yield estimated from incomplete WC band recoveries is $\leq 10\%$. The fate of this product is unknown, and slow back-reaction to the WC structure or formation of other products are possible.[14] The 1720 cm^{-1} product band shows a linear UV power dependence, and no build-up of other photoproducts was observed. Hence multi-photon-induced photochemistry can be excluded (see Sections S16 and S17 for an extended discussion).

For a global data analysis of the transient signals, we employed the kinetic model described in Section S7. The bleach recoveries (Figure 3d) and the decays of the hot bands (Figure 3e) are well described by a single exponential function with a time constant $\tau_{vc,G\cdot C} = 7.2 \pm 0.1$ ps associated with the cooling of vibrationally hot S_0 G·C molecules in chloroform. The product band at 1720 cm^{-1} (Figure 3c) was modeled with a consecutive reaction scheme,

$$G[-H]\cdot C[+H] \xrightarrow{\tau_{biradical}} G^*\cdot C^*(S_0, \nu > 0) \xrightarrow{\tau_{vc,G^*C^*}} G^*\cdot C^*(S_0, \nu = 0)$$

with $\tau_{biradical} = 2.9 \pm 0.2$ ps fixed from the TEAS measurements and $\tau_{vc,G^*\cdot C^*} = 5 \pm 2$ ps. The lifetime of the G[−H]·C[+H] biradical ($\tau_{biradical}$) is determined by its decay through a conical intersection returning it to the ground electronic state, whereas $\tau_{vc,G^*\cdot C^*}$ represents the vibrational cooling of the G*·C* tautomer in its electronic ground state.

Although the G[−H]·C[+H] biradical intermediate (**2**) has a lifetime of 2.9 ps, the CT state is populated within 40 fs. Hence the energy of the absorbed photon dissipates on timescales that may be competitive with formation of excimer states within a DNA single strand, which are precursors for DNA photodamage products, such as cyclobutane dimers or (6–4) adducts of pyrimidine bases.[15] However, the $\leq 10\%$ G*·C* (**3**) quantum yield in solvated, but isolated G·C WC base pairs (**1**) means that a considerable fraction of photoexcited G·C forms a potentially mutagenic tautomeric photoproduct. The characteristic G*·C* tautomer band at 1720 cm^{-1} was not observed in a recent TVAS study of natural calf thymus DNA following 266 nm excitation,[5b] but lifetime shortening to 40 ps of the G·C WC pairs was identified and attributed to interstrand proton transfer. The details of the interplay between horizontal and vertical interactions in double-stranded DNA, as well as the involvement of hydrogen bonding with water and proteins on the major and minor grooves in natural DNA therefore remain to be established, but collective observations hint at the importance of UV-induced EDPT pathways.

The mechanism that emerges from the above analysis is summarized in Figure 4. After excitation of G·C (**1**), ultrafast internal conversion from the $\pi_G\pi_G^*$ into a CT $\pi_G\pi_C^*$ state and subsequent proton transfer take place. This EDPT process forms the G[H]·C[+H] radical (**2**) with $\Phi_{biradical} \geq 0.6 \pm 0.1$ (for excitation at 260 nm), which is trapped in a minimum on the CT state for about 3 ps. At a conical intersection that connects the CT and S_0 states, an electron is transferred back from C[+H] to G[−H], followed by a proton transfer. This proton transfer occurs predominantly along the central $N_{1,G}$–H···N_C hydrogen bond, restoring the initial WC structure. However, up to 10% of the overall excited-state population undergoes a proton transfer along the $N_{4,C}$–H···$O_{6,G}$ hydrogen bond to form the long-lived ($\tau >$ 1 ns) G*·C* tautomer (**3**). Alternatively, the initially excited $\pi_G\pi_G^*$ state can be depopulated by monomer-like deactivation pathways with a quantum yield of $\leq 0.4 \pm 0.1$. These findings provide the most compelling experimental evidence to date for the involvement of EDPT-driven relaxation in a WC base pair and are consistent with the most recent comprehensive computational study by Sauri and co-workers on G·C base pairs.[12a] The authors concluded that the EDPT process is a likely deactivation mechanism that could compete with monomer-like deactivation pathways, and that it could be responsible for the formation of the G*·C* tautomer. The simulations predicted a timescale for the first hydrogen-atom transfer of 50 fs, which agrees well with our experimental determination of <40 fs. A clear understanding of the interplay between the interstrand and intrastrand dynamics remains to be established, but the fast (<40 fs) population of

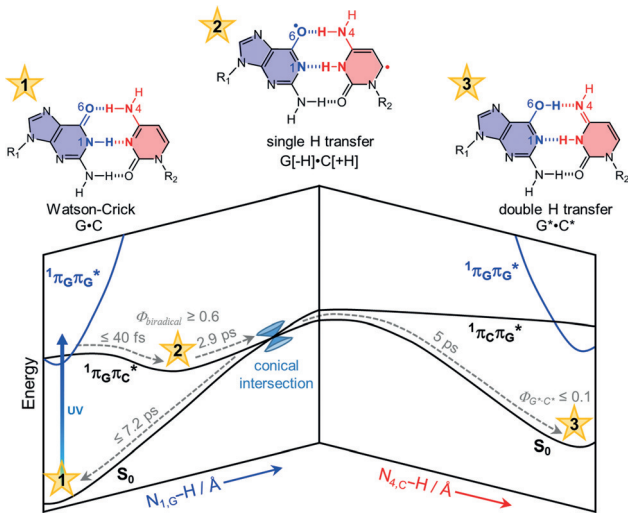

Figure 4. Schematic potential energy surfaces involved in the deactivation of the G·C Watson–Crick base pair (**1**) after UV absorption to a locally excited $\pi_G\pi_G^*$ state. EDPT within ≤40 fs produces the G[−H]·C[+H] biradical (**2**), which is trapped in a local minimum on the excited $\pi_G\pi_C^*$ charge-transfer PES and has a lifetime of 2.9 ps. After crossing the conical intersection, the majority of the molecules return to the S_0 state of the Watson–Crick structure by back-transfer of the electron and the central proton. The subsequent vibrational cooling of the G·C molecules on the S_0 PES is completed in 7.2 ps. A fraction of the G[−H]·C[+H] biradicals instead undergoes EDPT along the $N_{4,C}$–H···$O_{6,G}$ bond, leading to the G*·C* tautomer (**3**), which is stable for >1 ns. Monomer-like deactivation pathways are not shown.

CT states leading to interstrand EDPT deactivation and tautomerization is shown to be fast enough to compete effectively with monomer relaxation and excimer photochemical pathways. Although the present measurements do not confirm the participation of purely interstrand EDPT in more complex DNA duplexes, our work encourages greater consideration of this mechanism in future analysis of UV-induced photodynamics in double-stranded and higher-order DNA architectures.

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