JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Excited State Proton Transfer Is Not Involved in the Ultrafast Deactivation of Guanine−Cytosine Pair in Solution

Lars Biemann,[†] Sergey A. Kovalenko,[*,‡] Karl Kleinermanns,[*,†] Rainer Mahrwald,[‡] Morris Markert,[‡] and Roberto Improta[*,§]

[†]Department of Physical Chemistry, Heinrich-Heine University, University-str. 1, D-40225 Düsseldorf, Germany

[‡]Department of Chemistry, Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, D-12489 Berlin, Germany

[§]Istituto Biostrutture e Bioimmagini-CNR, Via Mezzocannone 16, I-80134 Napoli, Italy

Supporting Information

ABSTRACT: Different derivatives of Guanine (G) and Cytosine (C), which sterically enforce the Watson−Crick (WC) conformer, have been studied in $CHCl_3$ by means of broad-band transient absorption spectroscopy. Our experiments rule out the involvement of an Excited State Proton Transfer (ESPT), which dominates the excited state decay of GC in the gas phase. Instead, the ultrafast dynamics via internal conversion occurs in a polar environment mainly by relaxation in the monomer moieties. Time-dependent density functional theory (TD-DFT) calculations in solution indeed indicate that population transfer from the bright excited states toward the charge transfer state is not effective in $CHCl_3$ and a noticeable energy barrier is associated with the ESPT reaction. ESPT is therefore not expected to be a main deactivation route for GC pairs within DNA.

One of the most popular mechanisms for the fast and effective excited state deactivation of DNA (limiting potentially dangerous mutagenic events)[3,4] involves interbase proton transfer.[1−5] For cytosine(C)−guanine(G) Watson−Crick (WC) pairs in the gas phase, an intermonomer Excited State Proton Transfer (ESPT) reaction represents the main nonradiative decay path.[6−10] An easily accessible conical intersection (CI) connects the $^1\pi\pi^*$ spectroscopic states, mainly delocalized on guanine ($G\pi\pi^*$) and on cytosine ($C\pi\pi^*$), with an excited state with G → C Charge Transfer character (GC_{CT}). GC_{CT} is hugely stabilized by a $G^+ \rightarrow C^-$ PT reaction, involving the azine proton, ultimately leading to a diradical (GC_{dir}) state (G-H·)(CH·), and to an effective CI with the ground electronic state (S_0) (see Figure 1). The involvement of ESPT in the deactivation of GC pairs in solution[13−15] and in DNA[11,12,16−21] is instead matter of a very lively debate. The excited state decay of GC derivatives WC pairs in $CHCl_3$ is here studied by broad-band transient absorption spectroscopy, providing the complete spectral evolution of dark and bright excited states in the 270−700 nm range, and quantum mechanical calculations, ruling out the involvement of ESPT. Instead, the ultrafast dynamics occurs mainly by relaxation in the monomer moieties.

As shown by a thorough analysis of their stationary infrared spectra (see the Supporting Information (SI)), silylated tertiary butyl derivatives of G and C (see Figure 1 and Scheme 1 in the SI) sterically enforce the WC conformer in $CHCl_3$. Using an initial concentration $c_0 = 1 \times 10^{-3}$ mol/L like in the femtosecond experiments gives a cytidine solution that consists of 93% monomeric units, while in the guanosine solution, 56% of the molecules are present as monomers. After mixing these solutions, over 80% of the guanosine and cytidine molecules are incorporated in GC-dimers, only 2% of guanosine forms homoaggregates, and CC-dimers can be neglected. Our estimates are in good agreement with those previously obtained by the Temps group.[13,14]

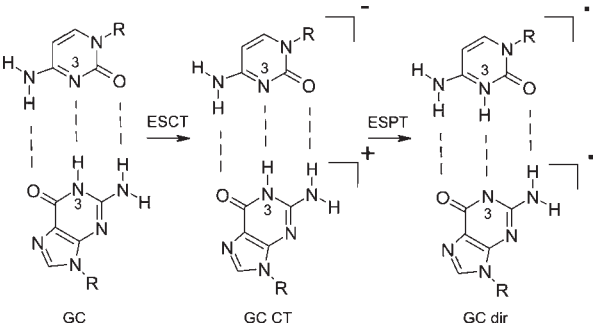

Figure 1. GC-Watson−Crick dimer (up), which undergoes a G → C CT reaction (central), followed by ESPT of a diradical species (bottom) very close to a CI with S_0. For theoretical calculations, methylated derivatives (R = CH_3) were used, while in the experiments, nucleosides with *tert*-butyldimethylsilyl-groups at sugar positions 2, 3, and 5 for guanosine (G3b) or at 3 and 5 for deoxyguanosine(G2b) were examined. If necessary for discrimination, we use G3b and G2b for G. Analogous nomenclature for cytidine.

The TA spectra of the isolated monomers (see Figure 2) provide a picture similar to that of the corresponding nucleotides in aqueous solution,[23] the small differences found for G being explained by the contribution of GG dimers (see SI for details). The careful analysis of the concentration dependence made by Schwalb et al.[13] indicates that excitation at 283 nm (near to our pump wavelength) leads to a somewhat faster decay of the fluorescence signal of GG aggregates than that of the monomers, mainly due to the smaller amplitude of a long-living (time constant >250 ps) component.[13]

In Figure 3, the TA spectrum of the GC dimer is compared with the simulation of the G + C mixture, computed simply as the sum of the spectra of the isolated monomers, shown in Figure 2. The two sets of spectra are very similar, both on the fast (<2 ps) and on the slower (2−100 ps) time scales: in the WC GC dimer,

Received: September 23, 2011
Published: November 10, 2011

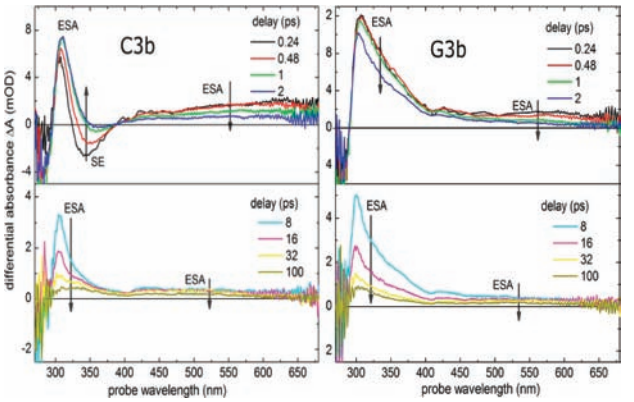

Figure 2. Transient absorption spectra of C3b and G3b in $CHCl_3$ upon excitation at 284 nm at early times (top) and later times (bottom), showing excited state absorption(ESA) and stimulated emission (SE).

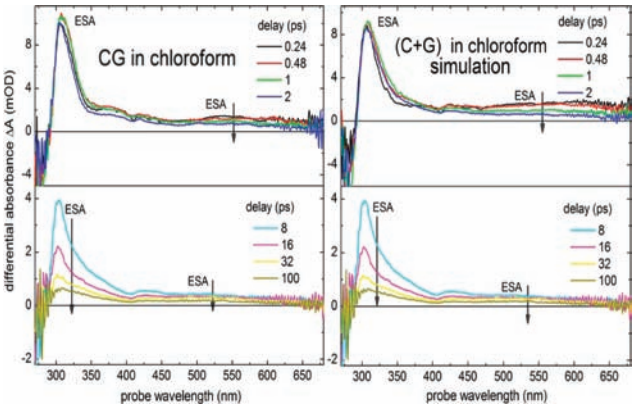

Figure 3. Spectral evolution of CG (C3bG3b) dimers in chloroform (left) and of the simulated spectra (right) calculated as the direct sum of the monomer C3b and G3b spectra in Figure 2 after excitation at 284 nm.

there is no signature of additional processes, which would qualitatively affect the excited state decay. Band integral analysis[22] (see Figure 4), focused on the two main features of the spectra (i.e., the Excited State Absorption at 300−350 nm and 400−680 nm), indeed indicates that the kinetics of the GC and the G+C systems are extremely close, the decay of the former systems being slightly faster at delay time >2 ps.

More in detail (see also SI), at 300−680 nm the main difference between monomer (G + C) and dimer (GC) spectra is at times later than a few picoseconds. In the range 0.2−1 ps, there is no significant difference between the G + C and GC curves. In the range 1−50 ps, the GC curve decays faster than the G + C curve in the range 300−350 nm and somewhat slower between 400 and 680 nm, see Figure 4. Typical decay times are 6.9/5.4 ps (300−350 nm) and 1.4/2.5 ps (400−680 nm) for G + C/GC, respectively. At 550 nm, the GC and the G + C decays can almost be superposed up to 8 ps and show a significant deviation only at later times, see Figure 5S in SI. A small long-term component with ESA decay up to 100 ps and probably longer can be identified in Figures 3 and 4.

In our broad-band TA measurements, there is therefore no experimental indication that additional ultrafast excited state decay routes (as ESPT in the gas phase) exist for the GC dimer

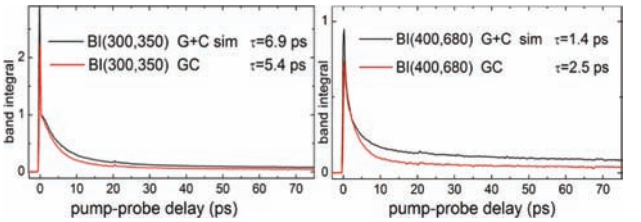

Figure 4. Temporal evolution of the band integrals of the GC (G3bC3b) dimer spectra in $CHCl_3$ (red) and of the simulated spectra calculated as the sum of the monomer spectra (black) for the spectral range 300−350 and 400−680 nm. Excitation at 284 nm.

in $CHCl_3$. On the ∼1 ps time-scale, the excited state decay of the GC dimer is very similar to that of G + C, while the formation of the dimer affects mainly the >10 ps time-scale, slower than that characteristic of the bright excited state decay of G and C. In this respect, our results are consistent with the fluorescence upconversion experiments by Temps and co-workers,[13] monitoring the kinetics of emission at 350 nm following excitation at different wavelengths, which indicate that the main difference between GC and G + C concerns the presence of very persistent time-components in the G monomer, whose lifetime spans the range 200−1400 ps, which is absent in GC. A long-living state (likely with $n\pi^*$ character), which could be involved[13] in the dynamics of G monomer, is expected to be destabilized by hydrogen bonding with C, giving account of the quenching of this very long living component in the GC dimer. Intermolecular hydrogen bonds could also modulate the PES of $G\pi\pi^*$ and $C\pi\pi^*$, affecting the height of energy barrier eventually present in the path toward the CI with S_0, explaining the small differences found in the ultrafast components for GC and G + C.

In a hydrogen bonding solvent like water, the excited state decay of guanosine monophosphate indeed does not exhibit ANY time-constant longer than 2 ps (since the $n\pi^*$ transitions are destabilized with respect to the $\pi\pi^*$ transition) and it is ultrafast (according to FU experiments, $\tau_1 \sim 0.2$ ps, $\tau_2 \sim 1$ ps[23]).

Accordingly, in a polar solvent, GC_{CT} is not predicted to be an important excited state decay route, although a partial population transfer from the spectroscopic states to GC_{CT} cannot be ruled out completely.

These indications are fully consistent with the predictions of Time Dependent (TD) DFT calculations in $CHCl_3$, including solvent effect by means of the Polarizable Continuum Model (PCM) model, in its accurate State-Specific implementation. The picture of the GC dimer decay provided by TD-CAMB3LYP (and by TD-PBE0; see SI) in the gas phase is extremely similar to what CASPT2/CASSCF and CC2 calculations show (see Table 1S and Figure 7S in the SI), especially with regard to the ESPT reaction. GC_{CT} is hugely stabilized by a barrierless PT reaction leading to a biradical state and, ultimately, to radiationless ground state decay. While GC_{CT}, $G\pi\pi^*$, and $C\pi\pi^*$ are very close in energy in the gas phase, facilitating population transfer from the spectroscopic states to GC_{CT}, in $CHCl_3$, the more polar GC_{CT} is instead significantly more stable than $G\pi\pi^*$ and $C\pi\pi^*$: at the FC point, GC_{CT} is more stable by ∼0.8 eV (SS-PCM/TD-CAMB3LYP/6-31G(d) calculations) than $G\pi\pi^*$, which corresponds to S_2.

Although a fully quantum dynamical study, rigorously including the coupling between the solute and the solvent degrees of

dx.doi.org/10.1021/ja2089734 |J. Am. Chem. Soc. 2011, 133, 19664–19667

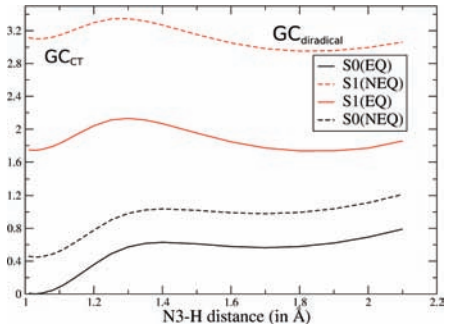

Figure 5. Energy of the GC_{CT} excited state for different values of the N3−H bond distance, as computed in $CHCl_3$ by SS-PCM/TD-CAM-B3LYP/6-31G(d) calculations on geometries optimized at the LR-PCM/TD-CAM-B3LYP/6-31G(d) level, by using the UA0 radii for building the PCM cavity.

freedom, would be very useful, in order to get a good estimate of solvent effect on ESPT, it is sufficient to explore two limiting situations, namely, the nonequilibrium (neq) and the equilibrium one (eq). In the former case, the ESPT process is assumed to be an ultrafast reaction, and the solvent does not have the time to reach the equilibrium with the GC_{CT} electron density. As a consequence, the slow degrees of freedom are always equilibrated with the S_0 electron density. In the eq limit, on the contrary, solvent is considered to adiabatically follow the motion of the N3−H proton, being always fully equilibrated with the GC_{CT} (or GC_{dir}) electron density.

As shown in Figure 5, $CHCl_3$ dramatically affects the features of the ESPT reaction (see also Figures 7S−9S in the SI). Unlike the gas phase, both at the neq and the eq level GC_{CT}-min and GC_{dir}-min have comparable energy. Furthermore, the ESPT path is not barrierless anymore: the predicted energy barrier is ∼0.25 eV (neq limit) and ∼0.35 eV (eq limit). Finally, GC_{dir}-min is not as close to the CI with S_0 as in the gas phase; the energy gap is always ≥1 eV. Actually, equilibration of solvent degrees of freedom is predicted to be a critical factor to reach the CI with S_0 (at the neq level, the gap with S_0 is indeed larger than 2.5 eV). Since this latter process should occur in $CHCl_3$ on the picosecond time-scale, it is not likely that the GC_{dir}-min/S_0 decay is faster than the fluorescence decay of the bright excited state localized on the G and C monomers, whose lifetimes in $CHCl_3$ (for λ pump = 262 nm) are ∼500 fs.[13]

Even a moderately polar solvent, thus, dramatically affects the importance of the PT reaction for the deactivation process, since population transfer to the CT state is more difficult, and, especially, PT implies the quenching of the strong dipole moment of the CT state. These general chemical-physical effects could be operative in many other systems/processes, and they should be carefully considered when analyzing the possible involvement of ESPT, on the basis of experiments performed in the gas phase or in apolar environments.

Our finding that no ESPT process is involved in the ultrafast part of the excited state decay of GC pairs embedded in a polar environment is fully consistent with the experimental results on GC polynucleotides, which do not decay to the ground state faster than their components. Furthermore, the isotope effect observed for the excited state decay of the $d(GC)_9 \cdot d(GC)_9$ double strand (suggesting the involvement of a PT reaction) concerns a slow time constant (∼20 ps), whereas no isotope effect is found for the faster time constants.[16,17]

Our results, thus, support the hypothesis that in GC rich polynucleotides the excited state decay is likely ruled by the formation of intrastrand exciplexes.[16] The formation of a CT excited state, eventually followed by ESPT, should in these cases involve rather an intrastrand GC excimer, eventually responsible for the ultrafast fluorescence decay,[15,19] and not an interstrand exciplex.

■ ASSOCIATED CONTENT

Supporting Information. Experimental and computational details; additional time-resolved spectra (Figures 1S−5S) ; computed absorption spectrum and additional calculations of the ESPT PES (gas phase, different choices of the functional or of the PCM parameters, Figure 6S−10S). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
skovale@chemie.hu-berlin.de; kleinermanns@uni-duesseldorf.de; robimp@unina.it

■ ACKNOWLEDGMENT

The authors would like to thanks Prof. N. Ernsting (Humboldt-Universität zu Berlin), Prof. V. Barone (Scuola Normale Superiore-Pisa), and Dr. D. Markovitsi (CNRS) for very useful discussions. R.I. thanks MIUR (FIRB Futuro in Ricerca and PRIN 2008) for financial support.

■ REFERENCES

(1) Löwdin, P. O. *Rev. Mod. Phys.* **1963**, *35*, 724.
(2) Löwdin, P. O. *Mutat. Res.* **1965**, *2*, 218.
(3) Kryachko, E. S. *Int. J. Quantum Chem.* **2002**, *90*, 910.
(4) Middleton, C. T.; de La Harpe, K.; Su, C .; Law, Y. K.; Crespo-Hernández, C. E.; Kohler, B. *Annu. Rev. Phys. Chem.* **2009**, *60*, 217–239.
(5) Markovitsi, D.; Gustavsson, T.; Vayà, I. *J. Phys. Chem. Lett.* **2010**, *1*, 3271–3276.
(6) Schultz, T.; Samoylava, E.; Radloff, W.; Hertl, I. V.; Sobolewski, A. L.; Domcke, W. *Science* **2004**, *306*, 1765–1768.
(7) Sobolewski, A. L.; Domcke, W.; Hättig, W *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17903–17906.
(8) Sobolewski, A. L.; Domcke, W. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2763–2771.
(9) Nir, E.; Plützer, Ch.; Kleinermanns, K.; de Vries, M. *Eur. Phys. J. D.* **2002**, *20*, 317–329.
(10) Abo-Riziq, A.; Grace, L.; Nir, E.; Kabelac, M.; Hobza, P.; de Vries, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 20–23.
(11) Markwick, P. R. L.; Doltsinis, N. L. *J. Chem. Phys.* **2007**, *126*, 175102.
(12) Groenhof, G.; Schäfer, L. V.; Boggio-Pasqua, M.; Goette, M.; Grubmüller, H.; Robb, M. A. *J. Am. Chem. Soc.* **2007**, *129*, 6812–6819.
(13) Schwalb, N. K.; Michalak, T.; Temps, F. *J. Phys. Chem. B* **2009**, *113*, 16365–16376.
(14) Schwalb, N. K.; Temps, F. *J. Am. Chem. Soc.* **2007**, *129*, 9272–9273.
(15) Schwalb, N. K.; Temps, F. *Science* **2008**, *322*, 243–245.
(16) de La Harpe, K.; Crespo-Hernández, C. E.; Kohler, B. *J. Am. Chem. Soc.* **2009**, *131*, 17557–17559.
(17) de La Harpe, K.; Crespo-Hernández, C. E.; Kohler, B. *ChemPhysChem* **2009**, *10*, 1421–1425.
(18) Crespo-Hernández, C. E.; de La Harpe, K.; Kohler, B. *J. Am. Chem. Soc.* **2008**, *130*, 10844–10845.

(19) Miannay, F.-A.; Bányász, A.; Gustavsson, T.; Markovitsi, D *J. Am. Chem. Soc.* **2007**, *129*, 14574–14575.
(20) Vayá, I.; Changenet-Barret, P.; Gustavsson, T.; Zikich, D.; Kotlyar, A. B.; Markovitsi, D. *Photochem. Photobiol. Sci.* **2010**, *9*, 1193–1195.
(21) Doorley, G. W. *Ang. Chem., Int. Ed.* **2008**, *48*, 123–127.
(22) Kovalenko, S. A.; Schanz, R.; Henning, H.; Ernsting, N. P. *J. Chem. Phys.* **2001**, *115*, 3256.
(23) Karunakaran, V.; Kleinermanns, K.; Improta, R.; Kovalenko, S. A. *J. Am. Chem. Soc.* **2009**, *131*, 5839.