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Ultrafast Electronic Relaxation in Guanosine is Promoted by Hydrogen Bonding with Cytidine

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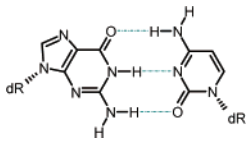
Base pairing by hydrogen bonding is the key feature determining the shape and function of DNA. It is responsible for the double helix structure, plays a role in the transcription of the genetic code into proteins, and enables the duplication of DNA molecules and thereby the reproduction of life. Yet the available information on the properties and the distinctive dynamics of H-bonded nucleobase pairs is still very limited. A number of multiphoton ionization and IR/UV double resonance spectra of H-bridged nucleobase complexes in the gas phase have been recorded in the past few years and provided valuable insight into different isomeric structures.[1−6] However, the observed resolved IR/UV spectra of adenine−thymine (A···T)[5] and guanine−cytosine (G···C)[2,6] belong to several non-Watson−Crick isomers, and the "canonical" Watson−Crick (WC) structures remained elusive. Only very recently has a very broad unstructured spectrum been tentatively attributed to the G···C WC complex,[6] the most stable G···C isomer. It was hypothesized that the broad bands might be indirect evidence for a significantly shorter excited electronic state lifetime of the G···C WC pair than for the other isomers,[6] as has been proposed by theoretical work.[7,8] However, broad unresolved spectral features can have many causes.

Here, we report on the results of the first femtosecond time-resolved experiment on the G···C WC base pair in solution to determine the effect of H-bonding on the excited electronic state lifetime.



Measurements were carried out using the technique of fluorescence up-conversion spectroscopy. We show that the formation of H-bonded pairs has profound impact on the ultrafast electronic dynamics. The results are of vital interest for bridging the huge gap between the well-known electronic properties of the isolated nucleobases and the strikingly different dynamics of DNA molecules.[9]

We investigated the G···C WC pair in chloroform (very dry spectral grade) by using (deoxy)nucleosides with bulky nonpolar *tert*-butyldimethylsilyl (TBDMSi) groups at the (deoxy)ribose units (G(TBDMSi)$_3$ and dC(TBDMSi)$_2$, referred to below as G and C).[10] The modified nucleosides are highly soluble in aprotic solvents, where the formation of H-bonded base pairs is strongly favored,[11,12] while stacked complexes, which are encountered in water, are unfavored. Furthermore, the dielectric constant ($\epsilon = 4.9$) of chloroform, which does not lend itself to H-bonding, is similar to that inside the DNA double helix ($\epsilon \approx 3-5$).[13] The ensuing association equilibria for the H-bonded homo- (G···G, C···C) and heterodimers (G···C) were analyzed by FTIR spectroscopy. A spectrum of the NH stretching regions displaying the formation of the G···C complex is shown in Figure S1. An analysis of the concentration-dependent band intensities by spectral fitting yielded association constants of $K_{C\cdots C} = 41(3)\ M^{-1}$, $K_{G\cdots G} = 730(180)\ M^{-1}$, and $K_{G\cdots C} = 6.4(1.6) \times 10^4\ M^{-1}$ for the respective dimers. Thus, G···C is by 2 orders of magnitude the most stable H-bonded complex. Moreover, the FTIR spectra showed no evidence for other main tautomers under the described conditions and indicated that essentially all G···C pairs have the WC conformation. The UV absorption spectrum of G···C was found to equate the superposition of the spectra of G and C (see Figure S2), and no exciplex-like fluorescence was found.

Our setup for fluorescence up-conversion spectroscopy has been described previously.[14,15] Briefly, the output of a home-built frequency-doubled non-collinear optical parametric amplifier at $\lambda = 283$ nm pumped by a Ti:Sa laser (Clark CPA 2001) was used to excite the sample in a flow cell with 1 mm path length. A second, time-delayed pulse from the Ti:Sa laser ($\lambda = 775$ nm) acted as gate. The concentrations of the nucleosides (G, C) were 0.1 mM. Measured time-resolved fluorescence decay profiles for C, G, and G···C are presented in Figure 1. The fluorescence was detected at $\lambda_{fl} = 350$ nm, approximately the maximum of the emissions. Each decay profile was fitted with a sum of decaying exponentials convoluted with a Gaussian for the instrumental response function (IRF). A biexponential decay behavior, with a fast sub-picosecond decay component τ_1 and a second longer-lived component τ_2 of several picoseconds, was found for all profiles except G, for which three exponentials were needed. A table of the fit results can be found in the Supporting Information.

The decay profile for C is given in Figure 1a. The sub-picosecond component, $\tau_{C,1} = 0.67(2)$ ps, corresponds very well to results found previously for cytidine in water (0.40−0.72 ps).[16−18] A small amplitude of ≈5% is contributed by a rather long-lived component of $\tau_{C,2} = 21(4)$ ps. This second component arises because $CHCl_3$, as the solvent used, is rather apolar ($\epsilon = 4.9$) and the molecular environment therefore resembles gas-phase conditions, where some excited-state population may be temporarily trapped in an nπ^* state.[9] Gas-phase experiments on cytosine at $\lambda = 267$ nm gave an excited-state lifetime of $\tau_{C,2} = 1.9$ ps.[19] At $\lambda = 283$ nm, we excite much closer to the S_1 origin, minimize the amount of excess vibronic energy, and therefore observe a weak prolonged second decay component.

Figure 1b shows the fluorescence decay curve of G. Three decay components were found, $\tau_{G,1} = 0.84(10)$ ps, $\tau_{G,2} = 7.0(1.0)$ ps, and $\tau_{G,3} = 500(200)$ ps. The $\tau_{G,1}$ resembles the values found for guanosine in neutral aqueous solution (0.46−0.69 ps)[16−18] and for guanine in the gas phase (0.36 ps)[19] with excitation at $\lambda = 267$ nm. The $\tau_{G,2}$ emerges because of the solvent and wavelength effects already described above for C. At $\lambda = 283$ nm, we excite G close to the local minimum of its first $\pi\pi^*$ excited state, where population may be temporarily trapped, while excitation at $\lambda = 267$ nm reaches the second $\pi\pi^*$ state much higher.[20,21] The very long-lived component $\tau_{G,3}$ contributes only with ≈3% to the overall profile.

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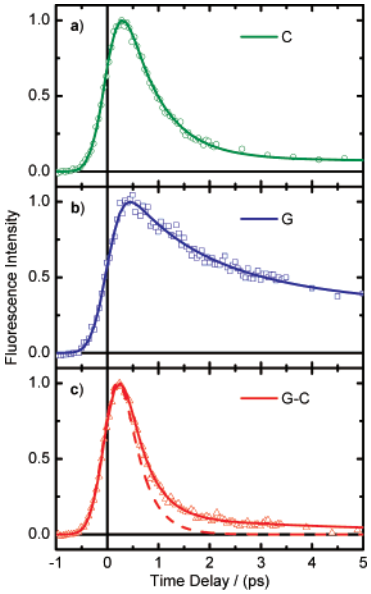

Figure 1. Fluorescence decay profiles of C (a), G (b), and G···C (c) in $CHCl_3$ ($c = 0.1$ mM for each nucleoside) after excitation at $\lambda = 283$ nm.

Thus, because of its very low amplitude and the fact that the experimental setup is designed for femtosecond experiments (max delay time = 900 ps), it could not be determined very accurately. A comparable value of ≈200 ps was found for guanosine in aqueous solution at pH = 2.[17] Eventually, we notice that there is ≈6% of G···G self-association at the concentration of 0.1 mM. This small amount was neglected at the present stage of the analysis.

Figure 1c exhibits the measured fluorescence time profile of the G···C base pair. It is eye-catching at the first glance that G···C shows the fastest decay. This becomes most obvious in comparison with G (Figure 1b). Biexponential fitting gave two decay constants, $\tau_{G\cdots C,1} = 0.42(3)$ ps and $\tau_{G\cdots C,2} = 5.0(1.0)$ ps. However, at the experimental concentration of 0.1 mM, we have only 68% of G···C dimer with 32% of free G and C. After correcting the experimental G···C profile for the contributions of the residual G and C in the solution, with appropriate account for the respective absorption coefficients of G and C at $\lambda = 283$ nm (Figure S2), we found that the "pure" G···C fluorescence profile is indeed determined by a single exponential decay function with a decay time of $\tau_{G\cdots C} = 0.355(3)$ ps. This decay is shown by the dashed curve in Figure 1c.

These results unambiguously demonstrate that WC base pairing reduces the excited-state lifetimes of C and G steeply. Comparing the different time profiles and lifetimes, the effect is even stronger on G than on C. We directly measured the ultrafast electronic relaxation of the G···C WC pair under well-defined conditions and showed that it is promoted by the intermolecular H-bonding. This effect was previously only assumed as a hypothesis to explain the very broad features in a gas-phase IR/UV spectrum.[6] We attribute the result to an optically dark doorway state in the G···C WC pair that mediates a faster relaxation than in the monomers.

Our findings can be explained in terms of a recently proposed coupled ultrafast electron−proton transfer mechanism that leads to a conical intersection of the photoexcited state with the electronic ground state.[7,8] This mechanism arises via a guanosine-to-cytosine $\pi\pi^*$ charge transfer (CT) state (excitation from the HOMO of G to the LUMO of C) that can only be energetically accessed in the WC form. The CT state is in close proximity to the S_1 minima of G and C, leading to a barrierless relaxation to the ground state. After photoexcitation and the charge transfer, a proton is spontaneously transferred along the central hydrogen bond from G to C, driven by charge compensation. After crossing the conical intersection of this CT state with the ground state, the G···C WC base pair re-forms its original structure by electron−proton back transfer. We found a much stronger impact of H-bonding on the relaxation dynamics of G, which accounts for the G-to-C mechanism. Very recently, Marwick and Doltsinis used nonadiabatic molecular dynamics simulations to calculate excited-state lifetimes of the G···C WC pair in the gas phase and in water and found biexponential decays with a very fast component of $\tau_1 \sim 0.03$ ps and a second component of $\tau_2 \sim 0.29$ ps.[22] Although τ_1 is beyond our time resolution, τ_2 is in very good agreement with our experimentally found value of $\tau_{G\cdots C} = 0.355$ ps.

Additional measurements on G···C and its constituents G and C at different concentrations, in different solvents, and at other excitation wavelengths are in progress. Furthermore, recent work on model DNA molecules containing A and T suggested that the electronic dynamics is dominated by intrastrand π-stacking interactions.[23,24] The present results demonstrate that, at least with G and C, interstrand coupling through H-bonds is likely to be important, as well. This underlines the need for further detailed studies of the electronic dynamics of small DNA building blocks, from the free bases and their H-bonded and π-stacked dimers to small oligonucleotides containing ≈4−8 bases, in order to rationalize the ensuing mechanisms in larger polynucleotides and DNA.

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Supporting Information Available: Experimental details, FTIR and UV spectra, and detailed fit results. This material is available free of charge via the Internet at http://pubs.acs.org.

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