Detection of the G(–H)• Radical in the Electronic Deactivation of the G–C Watson-Crick Base Pair

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Abstract: Transient absorption spectroscopy of the G–C base pair revealed the formation of the G(–H)• radical with lifetime 3 ps in the electronic deactivation. This radical is the key intermediate in an electron-coupled proton transfer.

OCIS codes: 300.6530, 350.5130.

1. Introduction

The absence of resolved vibronic structure in the electronic spectra of the guanosine–cytidine Watson–Crick (WC) base pair in the gas phase has been explained by an extraordinarily short lifetime of the excited $\pi\pi^*$ state due to an ultrafast G-to-C charge-transfer (CT) transition followed by a rapid proton transfer along the central (G)N–H···N(C) H-bond [1,2] and subsequent return to the electronic ground state through a conical intersection (CoIn). The existence of this process in G–C in solution, however, has remained highly controversial [3,4]. Here, we report the observation of a short-lived, distinctive, new excited-state absorption (ESA) band after UV excitation of G–C in $CHCl_3$ with pronounced absorption maximum at $\lambda \approx 390$ nm, which is not present in the excited-state spectra of G or C alone. By comparison with the known spectrum of the G(–H)• radical [5], this characteristic 390 nm feature provides clear evidence for the occurrence of the proposed electron-driven G-to-C proton transfer in photoexcited G–C.

2. Experimental Details

Guanosine–cytidine heterodimers with WC structure are formed as decidedly predominant hydrogen-bonded assemblies of the two nucleosides in solution in dry $CHCl_3$ [6]. The ultrafast dynamics in the base pair were thus studied after excitation at $\lambda = 260$ nm by femtosecond transient UV/vis absorption spectroscopy [7] of G resp. C in separate solutions and G + C under the same conditions in an equimolar mixture. To investigate the effect of increasing dimer concentration, each sample was measured at concentrations corresponding to optical densities of OD = 0.2 and 1.0. The recorded two-dimensional spectro-temporal maps of transient absorption vs. probe wavelength and time after excitation were then scaled according to concentration and relative fractions of absorbed UV pump light by the appropriate actinometric factors.

3. Results and Discussion

The two-dimensional absorption maps after excitation of G and C separately are given in Figs. 1a and b. No concentration dependence of the spectro-temporal shapes was observable for the samples in the investigated concentration range. The resulting synthetic superposition of the transient absorptions of G + C depicted in Fig. 1c is practically identical with the map for pure G, because the ESA by C is very weak. A moderately strong transient absorption maximum is visible around $\lambda \approx 340$ nm, followed by a weaker unstructured band at $\lambda \geq 400$ nm. In strong contrast, the measured transient absorption map belonging to the equimolar mixture of G and C in Fig. 1d showcases an intense additional, new ESA band with distinctive absorption maximum at $\lambda \approx 390$ nm next to less pronounced bands around 340 and 480 nm. At the experimental G and C concentrations of $c_0 = 5.6 \times 10^{-3}$ M used, the G–C WC dimer accounts for ≈ 93% of the dissolved molecules in the mixed solution. The "new" transient absorption in Fig. 1d around 390 nm therefore reflects a characteristic transition that is unique to G–C, but absent in G or C.

For further analysis, Fig. 1e depicts a plot of the difference spectrum resulting from the G–C measurement (Fig. 1d) minus the synthetic sum spectrum of unbound G + C (Fig. 3c) at a selected delay time of $\Delta t = 2$ ps after excitation. Included in this plot is the known UV/vis absorption spectrum of the G(–H)• radical of Candeias and Steenken [5]. The striking resemblance of the two spectra strongly suggests the formation of the G(–H)• radical as short-lived transient during the electronic deactivation of the G–C WC pair. Evidently, a sizable fraction of the G(–H)•–C(+H)• biradical

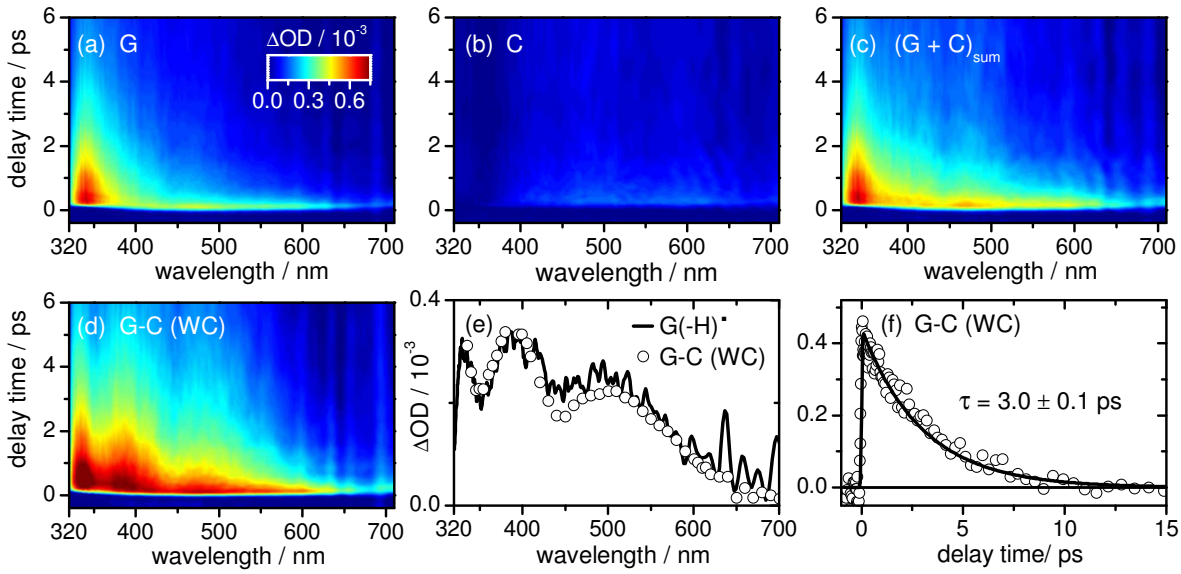

Fig. 1. Transient absorption maps after 260 nm excitation of a) G, b) C, c) G+C as synthetic superposition, and d) the measured equimolar mixture of G and C (93% G–C) in $CHCl_3$. Each absorption map is shown with the same intensity scale. e) Transient difference spectrum at $\Delta t = 2$ ps (black line) and normalized spectrum of G(–H)• (circles, taken from [5]). f) Time profile of the difference signal at $\lambda = 400$ nm together with its least-squares fit.

intermediate formed via the unique electron-driven proton transfer deactivation route in G–C [2] gets trapped for a short time dynamically or in a shallow potential energy well. The analysis of the temporal evolution of the difference spectrum afforded a lifetime of $\tau = 3.0 \pm 0.1$ ps (cf. Fig. 1f). Transient absorption by the C(+H)• counterpart appears to be weak, similar to the absorption of electronically excited C itself.

In conlusion, the close resemblance of the characteristic, G–C-specific transient absorption spectrum after electronic excitation with the spectrum of the G(–H)• radical provides strong support for the deactivation of the photoexcited base pairs via the proposed electron-driven proton transfer pathway. In this light, the reported ultrashort ($\approx$ 300 fs) fluorescence lifetime of G–C [3] may be attributed more likely to the transition from the initially excited state to the "optically dark" CT state rather than to the return of the excited dimer through the CoIn to the electronic ground state.

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