VIP DNA Photochemistry Very Important Paper

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Watson–Crick Base Pairing Controls Excited-State Decay in Natural DNA**

Dominik B. Bucher, Alexander Schlueter, Thomas Carell, and Wolfgang Zinth**

Abstract: *Excited-state dynamics are essential to understanding the formation of DNA lesions induced by UV light. By using femtosecond IR spectroscopy, it was possible to determine the lifetimes of the excited states of all four bases in the double-stranded environment of natural DNA. After UV excitation of the DNA duplex, we detected a concerted decay of base pairs connected by Watson–Crick hydrogen bonds. A comparison of single- and double-stranded DNA showed that the reactive charge-transfer states formed in the single strands are suppressed by base pairing in the duplex. The strong influence of the Watson–Crick hydrogen bonds indicates that proton transfer opens an efficient decay path in the duplex that prohibits the formation or reduces the lifetime of reactive charge-transfer states.*

Exposure to UV light is a major cause of damage to the genetic information of living organisms. UV radiation populates reactive excited states in the nucleobases. These excited states give rise to photochemical reactions that modify the molecular structure of DNA, thereby leading to mutations and cell death.[1] An understanding of the photophysical primary processes is consequently essential for deciphering the pathways that cause radiation-induced damage of the genetic code. The photophysics of single nucleotides is well understood. The absorbed photon energy is dissipated in an ultrafast internal conversion to heat, a process which minimizes the occupation of reactive excited states that could lead to DNA damage.[2] This deactivation mechanism was of utmost importance during the early stages of evolution when the genetic code developed under extreme ultraviolet irradiation. Today, nucleotides act as information bits in all organisms on earth. They are organized in long DNA double strands with a double helical structure held together by two major interactions—base stacking and base pairing. Both influence the photophysical properties of DNA. π stacking is known to be the reason for the formation of long-lived excited states with high yields after UV excitation. These states do not exist in the monomers.[3] Recently, these long-lived states were characterized in single strands. It was shown that they are formed in response to charge separation and charge delocalization.[4] Neutral excimers are discussed for homogeneous sequences.[5] In double-stranded DNA, base pairing is the second important interaction next to base stacking. It is unknown how interbase hydrogen bonds influence the photophysical behaviour of DNA but theoretical calculations suggest that they may provide an alternative ultrafast deactivation channel for excited states.[6] In this model, a charge-separated state within a base pair is formed, which decays ultrafast to the ground state through proton transfer between the hydrogen-bonded bases. Indeed, model base pairs in the gas phase[7] and isolated GC base pairs dissolved in chloroform[8] decay faster than the corresponding monomers. Interestingly, gas-phase experiments on different GC base pair structures yielded ultrafast excited-state decay only for the Watson–Crick arrangement, which is in agreement with theoretical studies.[9] In GC duplexes in aqueous solution, a quenching of the originally excited ππ* state has been found.[10] However, an accelerated return to the ground-state could not be shown. On the contrary, as in single strands, longer-lived excited states were observed.[11] As a consequence, current models to explain excited-state decay in the duplex argue that base stacking is the controlling interaction.[3a,b,4b,12]

Besides a few measurements on natural systems,[13] previous time resolved experiments were predominantly performed on synthetic oligonucleotides by using UV/Vis or fluorescence spectroscopy.[3a,11d,14] In this spectral range, the overlap of the absorption bands prevents the resolution of the contributions of individual nucleobases in the excited states. To allow the discrimination of the four bases, we used time-resolved IR-spectroscopy.[4a,15] The nucleobases give narrow and characteristic absorption bands in the mid-IR region, which allows the dissection of the individual contributions of the four DNA bases to the excited states of duplex DNA. By using UV excitation and IR probing, we are able to record the decay of the excited states after UV irradiation for each of the four bases in natural calf thymus DNA.

Marker bands for the four individual nucleobases in double-stranded DNA were identified by FTIR on the basis of literature data[16] (Figures S1–3 in the Supporting Information). These marker positions for each nucleobase are displayed in all of the figures as colored bars. The experiments were performed in D_2O buffer solution (see the Supporting

[*] D. B. Bucher, A. Schlueter, Prof. Dr. W. Zinth
BioMolecular Optics and Center for Integrated Protein Science (CIPSM), Ludwig-Maximilians-Universität München
Oettingenstrasse 67, 80538 Munich (Germany)
E-mail: wolfgang.zinth@physik.uni-muenchen.de
D. B. Bucher, Prof. Dr. T. Carell
Center for Integrated Protein Science at the Department of Chemistry, Ludwig-Maximilians-Universität München
Butenandtstrasse 5–13, 81377 Munich (Germany)
E-mail: thomas.carell@cup.uni-muenchen.de

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Information). In the experiment shown in Figure 1, we nonselectively excited the nucleobases of double-stranded calf thymus DNA at 266 nm and monitored the absorption changes in the mid-IR region. Upon excitation, the absorption bands of the original ground state disappear. We used these marker bands to identify the return to the ground state for each nucleobase. Figure 1 a shows the absorbance-difference spectrum at a short delay time (0.25 ps) when the initially excited electronic states of the nucleobases are populated. There is a broad absorption increase known to represent the absorption of the initially excited electronic state. Superimposed are narrow negative bands (bleaching). The bleaching of the signal matches the absorption of the ground state (Figures S1–3) and allows the assignment of the transient bands to the different nucleobases (Figure 1 a). The absorption change because the initially excited electronic state decays within less than one picosecond, thus leading to features of a vibrationally hot ground state. The cooling of this vibrational hot state is finished within the next 10 ps (time constant ca. 6 ps, Figure S4). The contour plot in Figure 1 b shows the evolution of the absorption-difference spectrum after the cooling process ($t > 10$ ps). The absorption-difference spectrum displays spectral features remarkably different to the ones observed directly after UV excitation of the DNA, and importantly, the spectrum changes considerably over time. The kinetics shown here evolve on the ten- to hundred-picosecond timescale in a manner similar to the data obtained for single-stranded DNA.[3a,4a] The long-lived states visible in Figure 1 b have amplitudes which amount to about 50 % of the initial bleached signal. The major feature of these long-lasting absorbance changes in calf thymus DNA show the following characteristic properties: First, the long-lived absorption-difference spectra differ from the bleached signals observed at short delay times. Second, the dynamics of the slow absorbance changes can be qualitatively modeled by two time constants ($\tau_1 = 40$ ps, $\tau_2 = 210$ ps). Given the complexity of the natural DNA, these time constants are within the range of previous publications.[3a,17] The present IR experiments follow transient species populated to a significant percentage (ca. 50 %), whereas long-lived transients with small relative amplitude, such as those found with emission measurements, are not addressed here.[13a] Third, and most important, are the spectra associated to the two long decay times (Figure 1 c). The two decay spectra $D_1(\nu)$ and $D_2(\nu)$ show clear differences. In the 40 ps decay spectrum, marker bands for the bases cytosine (C) and guanine (G) are dominant, while the 210 ps decay spectrum shows predominant contributions from adenine (A) and thymine (T). These spectral differences were also obtained when we subtracted spectra measured at late times from those measured at early times. This method excludes fitting artifacts (Figure S5).

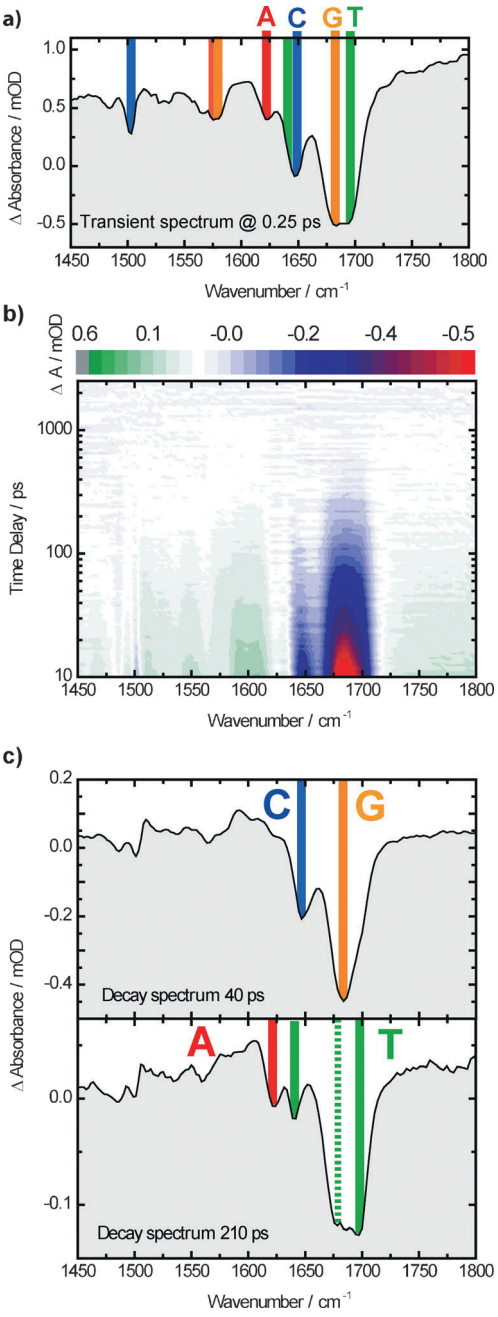

Figure 1. a) Transient difference IR spectrum at 0.25 ps after excitation at 266 nm. The marker band positions of the nucleobases Adenine (A), cytosine (C), guanine (G), thymine (T) are marked with coloured bars. b) Absorbance-change data at longer delay times in a contour plot. The cooling procedure before 10 ps is omitted. c) Decay-associated spectra $D_1(\nu)$ and $D_2(\nu)$ of slowly decaying species associated with the decay constants $\tau_1 = 40$ ps and $\tau_2 = 210$ ps.

If the long-lived states in the double-stranded system are assumed to be intrastrand charge-transfer states, one would expect a complex decay scheme. Different base sequences should give rise to different decay times since the recombination of charge-separated states is determined according to the Marcus theory by the redox difference between the involved bases.[3c] Such behavior has indeed been observed in single-stranded DNA. One would consequently expect a complex decay pattern in line with the extreme sequence heterogeneity of natural calf thymus DNA. For example, the lifetime of the GA exciplex is known to be around 300 ps,[4a] which is much longer than the observed times in the

DNA duplex. It is this joint decay of G and C on the one side and of A and T on the other that lead us to conclude that Watson–Crick base pairing and not base stacking controls the lifetime of the excited states.

The involvement of interstrand base pairing was further investigated in defined single- and double-stranded oligonucleotides. At first, we designed two complementary single strands with nucleotides selected in such a way that for each strand, one specific base, namely 2′-deoxyguanosine (G) or 5-methyl-2′-deoxycytidine (^{m}C), could be selectively excited by UV light at 295 nm (Figure S6).[4a] Investigation of these two DNA single strands (U^{m}CUUUUUU, AAAAAAGA; Figure 2a) showed long-lived excited states with marker bands

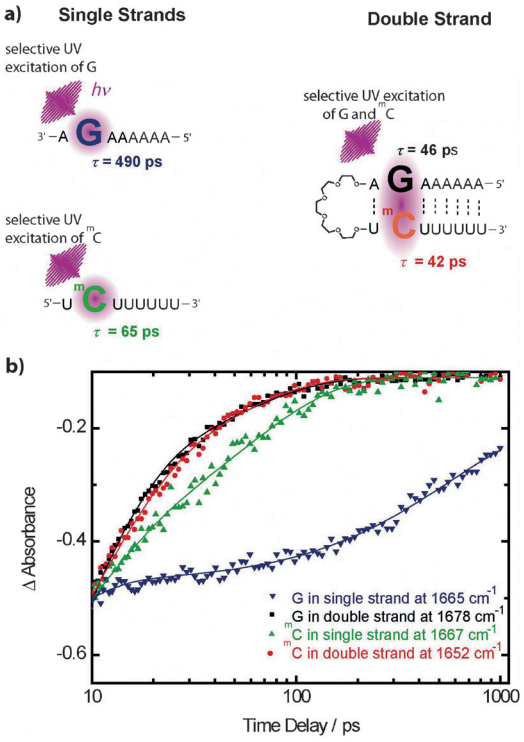

Figure 2. a) Picosecond pulses of UV light at 295 nm allow selective excitation of the 2′-deoxyguanosine (G) residue in the AAAAAAGA and the 5-methyl-2′-deoxycytidine (mC) residue in the UmCUUUUUU oligonucleotide. In the corresponding double-stranded hairpin AAAAAAGA·UmCUUUUUU, only G and mC are excited. b) Time-dependent absorption changes in the IR spectrum (normalized) recorded at the marker band positions of G and mC for the single- and double-stranded samples. The transients are fitted with a sum of exponentials (lines).

for charge-separated states (Figure S7). The charge-separated state $^{m}C^{\bullet+}U^{\bullet-}$ decays in U^{m}CUUUUUU with a lifetime of 65 ps. The charge-separated state $G^{\bullet+}A^{\bullet-}$ in AAAAAAGA vanishes with a lifetime of 490 ps. The temporal progression can be clearly seen by the positions of the respective ground-state bands of mC and G (Figure 2b, green and blue, respectively). The results fully agree with previous data obtained with other single-stranded DNA.[4a] We next prepared a duplex with the same sequence. To obtain a stable structure, we connected the strands through a hexaethylene glycol linker (Figure 2a) to give a hairpin structure (Figure S8, S9). In this duplex, the mC and G residues were selectively excited and the ground-state recovery of the mC and G bands in the duplex could be monitored separately as a result of their distinct absorbance bands in the mid-IR region. The transients at 1678 cm^{-1} (marker band for G; black) and 1652 cm^{-1} (marker band for ^{m}C; red) in the double-stranded hairpin are plotted in Figure 2b. It is evident that the decay of the long-lived excited states of ^{m}C and G is much faster in the duplex than in the corresponding single strands. More importantly, we observe very similar time constants of ca. 40 ps for the decay of both ^{m}C and the G in the duplex, whereas in the single strands, the excited states of these bases decay much more slowly with vastly different time constants. This effect cannot be caused by structural differences between the single- and the double-stranded DNA since charge-transfer states are nearly independent of base-stacking geometry.[18] The decay of the excited states of G and ^{m}C are thus coupled in the duplex and it is this coupling that blocks formation of the reactive radical-type charge-transfer states. Furthermore, the time constant for the GC pair in the artificial hairpin is the same as in natural calf thymus DNA. These results show directly that the hydrogen bonds of the base pairs control the formation and decay of the long-lived excited states. Our data suggest that interstrand proton transfer initiated by photoexcitation of the DNA is the reason for the concerted decay of paired bases. Indeed, proton transfer between paired bases caused by intrastrand charge transfer after UV excitation has been postulated in the literature based on hydrogen/deuterium isotope effects.[3a,19] The observed concerted decay of excitation in paired bases could also be explained by a model consisting of interstrand charge transfer coupled to proton transfer.[6] However, the long time constants observed in the present experiments stand in contrast to the proposed subpicosecond decay. Besides these proton transfer processes induced by charge transfer, a mechanism involving double proton transfer induced directly by the electronic excitation could explain the observations.[20] As a common feature of these models, the excited state decay is related to interstrand proton transfer and the observed changes in the IR-absorption spectrum originate from the modified arrangement of hydrogen bonds in the base pairs.

The molecular processes following the excitation of the nucleobases are illustrated in Figure 3. In monomers, the optically excited ππ* state mostly decays fast by internal conversion to the ground state. In single strands, base stacking enables photochemical reactions between neighboring bases (e.g., to give a cyclobutane pyrimidine dimer (CPD)[15b,c] or a 6–4 lesion[15a]) and gives rise to a considerable number of long-lived radical-pair states. These charge-separated states are precursors of the harmful 6–4 lesion in DNA[21] and possibly induce oxidative as well as reductive damage.[22] Because base stacking enables the formation of charge-transfer states, it is the stacking which is responsible for DNA damage (Figure 3). Base pairing through Watson–Crick hydrogen bonds opens up new decay channels through proton transfer[23] and may deactivate dangerous charge-transfer states.

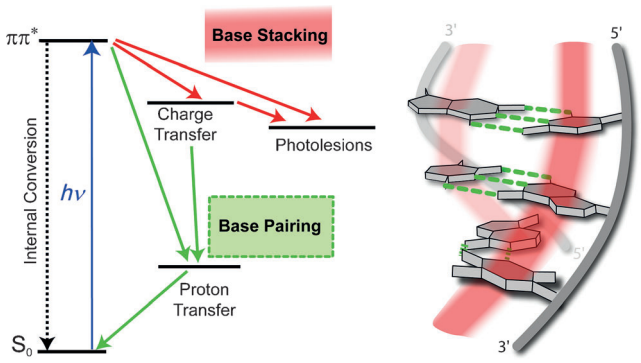

Figure 3. Base stacking in the DNA strands supports the ordered arrangement of bases, which is a prerequisite for many of the functions of DNA. However, the stacking also leads to the formation of photolesions, either directly from the originally excited ππ* state or through charge-separated intermediates. Base pairing between the bases of two complementary strands opens up a new decay channel which deactivates the ππ*[10] and charge-separated states. Base pairing thus counteracts the destructive action of charge-separated states, thereby supporting the integrity of the genetic information.

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