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Identification of charge separated states in thymine single strands†

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UV excitation of the DNA single strand (dT)₁₈ leads to electronically excited states that are potential gateways to DNA photolesions. Using time-resolved infrared spectroscopy we characterized a species with a lifetime of ~100 ps and identified it as a charge separated excited state between two thymine bases.

The formation and decay of electronically excited states in DNA has been of major interest since the pioneering work on DNA photochemistry in the 1960s.¹ Based on the finding that the integrity of DNA is endangered by the mutagenic potential of UV induced photolesions,^{2,3} attempts to unravel the relevant damage pathways are directly bound to monitor the initial steps after UV absorption. Recent advances in time resolved techniques made it possible to follow DNA excitations in real time.^{4,5} It could be shown that single bases and mononucleotides predominantly decay to the electronic ground state *via* ultrafast internal conversion. In base sequences and double stranded DNA increasing contributions of longer lived excited states were detected.⁵ Thereby, excitons, exciplexes and excimer states with charge transfer character have been proposed to be responsible for prolonged excited state lifetimes.^{6–9}

The most abundant UV induced DNA lesions occur between thymine bases, and all-thymine single strands became the focus of a series of time resolved experimental and computational studies.^{10–17} Thereby, charge transfer (CT) states have been suggested to act as precursor species for the formation of (6-4) photoproducts in thymine sequences.¹⁷ Yet, until now charge transfer excited states between two thymine bases have not been

identified unequivocally. Moreover, earlier experiments in the UV and visible spectral range are not conclusive. Reaction dynamics on the 100 ps time scale have been observed in two independent studies on (dT)₁₈ and (dT)₂₀.^{14,18} In one case the decay was tentatively assigned to a singlet nπ* state.¹⁸ In the other case the decay was ascribed to the quenching of triplet states eventually leading to the formation of cyclobutane pyrimidine dimer (CPD) lesions.¹⁴ Neither of the studies reported direct evidence for the interpretations due to the lack of conclusive spectral signatures in the UV and visible spectral range monitored in these experiments.

In this study, we applied time resolved IR spectroscopy (TRIR) to reinvestigate the picosecond dynamics in the all-thymine single strand (dT)₁₈. TRIR has been shown to allow the unequivocal spectral identification of electronically excited states, reaction intermediates and photoproducts.^{8,13,19–21} The obtained data show that UV excitation in (dT)₁₈ leads to the formation of electronically excited states that decay to the electronic ground state on a 100 ps time scale. Comparison of the spectral signature with experimentally determined anionic and cationic thymine species provides unequivocal evidence that the observed species resemble charge separated states between two thymine bases. The latter are formed with a quantum yield of ~0.07.

Experimental data obtained for (dT)₁₈ in buffered D₂O solution after excitation at 266 nm are given in Fig. 1. To emphasize the kinetics on the 100 ps time scale the data have been corrected for absorption changes on the nanosecond time scale. In this procedure, experimental data between 400 ps and 600 ps were averaged and subtracted from the original data set (see ESI†). The contour plot in Fig. 1 shows bleach signals (indicated in blue) at 1630 cm⁻¹, 1664 cm⁻¹ and 1696 cm⁻¹ matching the ground state absorption spectrum. Prominent positive absorption bands are located at 1570 cm⁻¹ and 1760 cm⁻¹ (indicated in red). The decay of the excited state absorption and the recovery of the ground state at 1664 cm⁻¹ both occur on the 100 ps time scale. Representative time traces at 1570 cm⁻¹ (excited state absorption, red) and 1664 cm⁻¹ (ground state bleach, blue)

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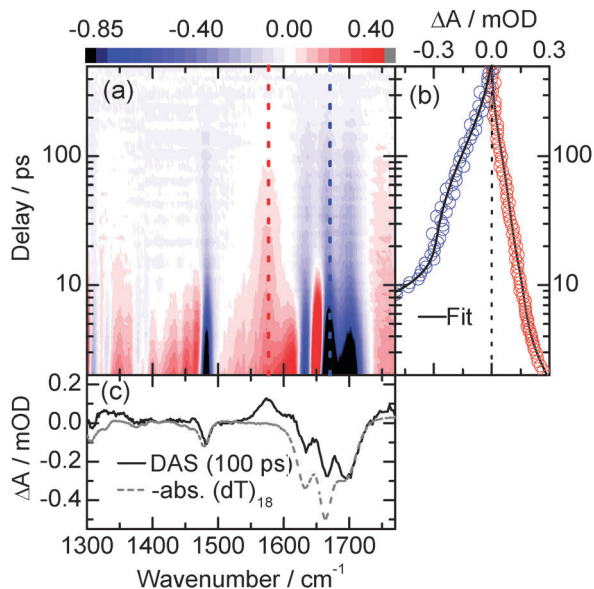


Fig. 1 Time resolved IR absorption changes of $(dT)_{18}$ after 266 nm excitation. (a) Contour representation of the transient absorption difference spectra corrected for the absorption changes at late delay times (see text for details). (b) Time dependence of the absorption changes (circles) recorded at 1570 cm^{-1} (red) and 1664 cm^{-1} (blue). Results of a global fitting procedure using a sum of two exponentials are given as solid lines. (c) The spectrum (DAS) associated with the 100 ps component obtained by this fit is given by the black solid line. The inverted ground state absorption spectrum of $(dT)_{18}$ is given as broken line for comparison.

underscore this assignment (Fig. 1b). The analysis of the data set relied on a global fitting routine.²² Thereby, a sum of exponentials convoluted with the experimental response served as trial function. The experimental data can be readily reproduced by a fast component representing signatures on the femtosecond to picosecond time scale including ultrafast internal conversion and cooling kinetics.^{13,23,24} Additionally, a slow decay component with a lifetime of ~ 100 ps is observed. The decay associated spectrum DAS(100 ps) of the latter is given in Fig. 1c together with the inverted ground state absorption of $(dT)_{18}$ (broken line) for comparison.

The nature of the species associated with the 100 ps decay component DAS(100 ps) will be discussed next. In a transient absorption study by Kwok *et al.* a species with a similar lifetime of ~ 140 ps was found for $(dT)_{20}$ in aqueous solution and interpreted as triplet decay.¹⁴ Yet, a recent study on $(dT)_{18}$ covering the nanosecond time scale excludes this interpretation.²⁵ The latter study revealed that the lowest triplet state ^3LT of thymine exhibits a characteristic spectral signature with a triplet marker band around 1600 cm^{-1} that decays on the 10 ns time range. A comparison of the decay associated spectra of the triplet state with DAS(100 ps) is given in Fig. 2. Clearly, the signature of the triplet state (indicated by the red line) is missing in the DAS(100 ps). Based on the spectral signature and the different time scales an assignment to the lowest triplet state can be ruled out for the DAS(100 ps). Also the assignment to a triplet $n\pi^*$ state is unlikely.

The latter would be expected to serve as gateway to the lowest triplet state with $\pi\pi^*$ character. Yet, as can be seen from

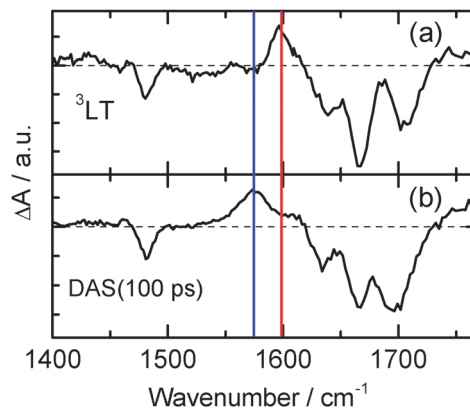


Fig. 2 Comparison of absorbance changes indicative of the decay of the lowest triplet state ^3LT (a) and of the decay component DAS(100 ps) (b) obtained for $(dT)_{18}$. The position of the marker band for the lowest triplet state around 1600 cm^{-1} is indicated in red, the characteristic absorption of the 100 ps decay component around 1575 cm^{-1} is indicated in blue.

the data presented in Fig. 1 such a state is absent and the observed state related with the DAS(100 ps) decays directly to the starting material.

In an earlier study Kohler *et al.* were the first to observe an electronically excited species with a lifetime of ~ 103 ps in the all-thymine single strand $(dT)_{18}$.¹⁸ The observed species was tentatively assigned to a singlet $n\pi^*$ state.¹⁸ Yet, the assignment to a singlet $n\pi^*$ state is unlikely in view of results obtained for TMP where a 100 ps decay component was not observed (see ESI†). This suggests that the 100 ps component is not an intrinsic excited state of the thymine base, but is related to the interaction of thymine bases in the strand. It should be noted that this result contradicts the study by Kohler *et al.*, where a 100 ps time constant was also observed for TMP.¹⁸ Yet, preliminary experiments by the present authors on the transient dichroism of thymine and TMP have shown that such a 100 ps component of TMP can be assigned to orientational diffusion.

Based on the assumption that the excited state is associated with base interactions, excimer or exciplex states have to be taken into consideration. We propose that the observed species is due to an excimer state with significant charge transfer character, respectively a charge separated state on adjacent or separated thymine bases in the strand. To support our hypothesis we compare the observed DAS(100 ps) with the IR signatures of the corresponding cationic and anionic thymine species (for details see ESI†). The cationic species $\text{T}^{\bullet+}$ was generated by two-photon ionization of $(dT)_{18}$ and its spectrum recorded by TRIR (Fig. 3a). Obtaining the anionic species $\text{T}^{\bullet-}$ requires a reducing agent. Photo-excited adenine bases reduce the thymine moiety in TpA in agreement with recent results by Doorley *et al.* on the dinucleotide ApT.⁶ Therefore, a TRIR experiment on photo-excited TpA yields the decay spectrum of $\text{T}^{\bullet-}\text{pA}^{\bullet+}$ (see ESI,† Fig. S6a). In order to obtain the information on $\text{T}^{\bullet-}$ the spectrum of $\text{T}^{\bullet-}\text{pA}^{\bullet+}$ must be corrected for the contribution of the cation radical $\text{A}^{\bullet+}$ (see ESI,† Fig. S6a). The latter cation signature was determined *via* two-photon ionization of $(dA)_{18}$ taking into account the specific

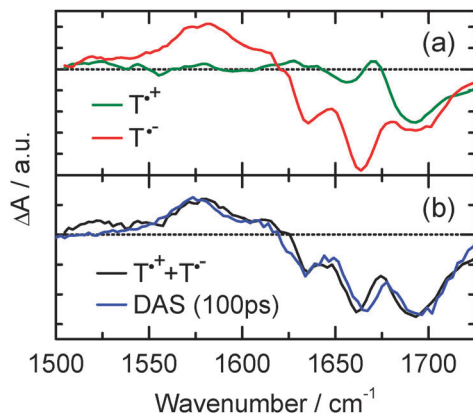


Fig. 3 (a) Signatures of the thymine cation ($T^{\bullet+}$) and anion ($T^{\bullet-}$) obtained for (dT)₁₈ in independent experiments. (b) Comparison between the charge transfer signature calculated from the cation and anion signals ($T^{\bullet+}+T^{\bullet-}$) and DAS(100 ps).

spectral changes due to base stacking interactions in (dA)₁₈ (see ESI†). The spectra of the cation $T^{\bullet+}$ and the anion $T^{\bullet-}$ obtained by this procedure were added to yield a prediction for the spectrum of the CT state. A direct comparison of this spectrum and DAS(100 ps) is given in Fig. 3b. The excellent match between the signatures for CT state (black line) and DAS(100 ps) (blue line) shows that DAS(100 ps) is indeed a CT state and that CT states are present in all-thymine sequences after UV excitation. An estimate of the quantum yield for CT formation results in a value of $\varphi_{CT} \sim 0.07$ (for details see ESI†).

From the lifetime of the primary excitation in thymine derivatives ($\tau_{fl} \approx 0.5\text{--}1$ ps see ref. 26) and the observed yield one can estimate that the rate constant for CT $k_{CT} \approx \varphi_{CT}/\tau_{fl}$ is of the order of 10^{11} s^{-1} . This value applies for a homogeneous situation where all excited thymines have the same probability for CT formation. For an inhomogeneous situation, *i.e.* when a geometry favourable for CT is realized only for a minority of thymines in the strand the reaction rate will be substantially higher. The charge recombination (CR) process occurs with a rate constant k_{RC} of 10^{10} s^{-1} . The order of magnitude of these rate constants is consistent with predictions from electron transfer theory (see ESI†). Electron transfer theory also suggests, that thymine in a triplet state – due to its lower energy – would allow only a very slow CT (at a rate constant of $4 \times 10^3 \text{ s}^{-1}$). This matches our recent findings that the triplet excited thymine in (dT)₁₈ exhibits a lifetime of 10 ns and decays *via* the formation of a biradical and not *via* a CT state.²⁵

In a recent quantum chemical study, CT states between thymine bases have been discussed to be the starting point for the formation of the (6-4) lesion.¹⁷ The decay of such a charge transfer state was predicted to yield oxetane intermediates, as precursors of the (6-4) adduct. Yet, as shown in Fig. 1a, we do not observe any other species formed with the decay of the CT states. Thus, the vast majority of the CT-states decays back to the electronic ground states of the starting material. However, since the quantum yield of (6-4) formation φ_{6-4} is known to be very low (on the order of 0.005),⁷ a similar small yield for formation of

the proposed oxetane intermediate might hamper its detection. The yield φ_{6-4} and the yield of the CT state φ_{CT} reported here imply that less than a fraction of 0.1 of the CT population transforms into (6-4) photolesions – provided that the CT state is indeed the precursor.

In conclusion, time resolved IR spectroscopy was used to investigate the 100 picosecond dynamics of the all-thymine single strand (dT)₁₈ after UV excitation. We observed the formation of an electronically excited species that decays with a time constant of ~ 100 ps. By comparison with the experimentally determined signatures of cationic and anionic species of thymine compounds the latter could unequivocally be assigned to a charge transfer (CT) state. While CT states have been demonstrated for mixed DNA sequences before, the present study yields the first direct experimental assignment of a CT state between two thymine bases on the 100 ps time scale. The latter is of major importance as CT states have been proposed to act as precursors for the formation of DNA photolesions. Yet, although the CT states are initially formed with a comparably high quantum yield of ~ 0.07 , we did not see any indication for the formation of reaction intermediates or photolesions. Instead, the CT states decay predominantly to the electronic ground state.

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