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Thymine Dimerization in DNA Model Systems: Cyclobutane Photolesion Is Predominantly Formed via the Singlet Channel

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Photoreactions induced by ultraviolet radiation are among the most important external hazards for the integrity of DNA.[1] The photolesion with the highest abundance is the [2 + 2] photoaddition of thymine bases adjacent on a DNA strand.[2] This photoaddition yields a cyclobutane pyrimidine dimer (CPD). The formation of the CPD lesion has first been described in 1960.[3,4] Nevertheless, the kinetics of its formation is still under debate. In solution the photodimerization of single thymine bases involves a triplet state.[5,6] A reaction via a singlet channel is precluded in this diffusion limited process since singlet excitations in thymine are too short-lived.[7,8] In DNA strands thymine bases are kept in proximity by the sugar−phosphate backbone and the diffusion limit does not apply. So in DNA the CPD lesion could in principle be formed via excited singlet or triplet states.[9]

In a recent femtosecond IR experiment[10] by some of the present authors evidence was given that in an all thymine DNA single strand ($(dT)_{18}$) the CPD lesion is formed in ∼1 ps. The experiment relied on highly characteristic IR marker bands in the fingerprint region detected as early as ∼1 ps after UV excitation. Excited electronic states other than the primarily excited singlet $\pi\pi^*$ state are much longer lived. This favors a concerted CPD formation from the $^1\pi\pi^*$ precursor. The formation time of ∼1 ps is shorter than the time scale of larger conformational changes in DNA single strands;[11] i.e., the DNA strand is essentially static during the dimerization. Based on these observations we have postulated[10] that the low quantum yield φ_D for CPD formation (∼3%[12] for $(dT)_{20}$) is due to the rareness of reactive conformations in a thermal ensemble. Molecular dynamics simulations on Thymidylyl(3′→5′)thymidine (TpT) are in favor of the rareness of reactive conformers[13] and suggest that the CPD yield should scale with their thermal population. Recent femtosecond pump−probe experiments in the UV/vis[14] question the ultrafast nature of the CPD formation and thereby the conformational control of the quantum yield. In the study the behavior of thymidine (dT) is compared with that of $(dT)_{20}$. In dT a spectroscopic species with a lifetime of ∼4 ns has been observed by transient absorption spectroscopy. In $(dT)_{20}$ this lifetime is reduced to 140 ps. The reduction is ascribed to the reactive quenching of the triplet state eventually resulting in CPD formation.

Here, we will show that in all thymine DNA models the CPD yield is settled after ∼1 ps and that this yield depends on the fraction of reactive conformers. To this end femtosecond IR experiments on three dimerizable samples and a reference sample (thymidine monophosphate, TMP) were performed. The three dimerizable samples and their respective quantum yields φ_D^{cw} (in brackets) were TpT (∼1−2%[12,13,15,16]), $(dT)_{18}$ (yield for the closely related $(dT)_{20}$ ∼3%[12,16]), and T_LpT_L (∼6 times that of TpT).[17] In T_LpT_L (LNA-T dimer)[17] the furanose moieties of the deoxyriboses are forced into a C3′ endo conformation by a methylene clamp. Thereby the propensity toward CPD formation increases by a factor of 6 with respect to TpT (depending on the value used for TpT this translates into a yield φ_D^{cw} of 6−12%).

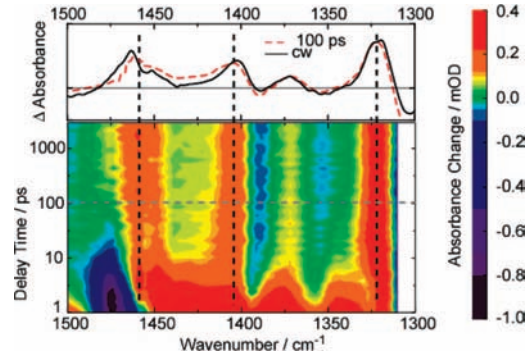

Figure 1. IR absorbance changes of T_LpT_L induced by UV-excitation. Top: The black line represents the difference spectrum obtained in a cw illumination experiment. Characteristic marker bands for the CPD formation are clearly visible. The red line stands for the difference spectrum recorded after 100 ps. Bottom: Contour representation of IR difference spectra for delay times between 1 ps and 3 ns. The vertical dashed lines trace the CPD marker bands back to ∼1 ps.

CW illumination of the three samples results in a growing in of IR bands in the 1300−1500 cm^{-1} range (exemplified for T_LpT_L in Figure 1, top). The spectral pattern matches the one obtained for an isolated CPD sample (see Supporting Information). The emerging bands are the aforementioned marker bands for CPD formation. The marker bands for each sample differ slightly, but they always allow a clear identification of the CPD lesion. In the femtosecond UV pump/IR probe experiment (experimental details are given in refs 10 and 18), the samples were excited with 300 fs UV pulses centered at 268 nm which is close to the peak of the absorption band of thymine.[19] Spectroscopic changes in the marker band region were probed by mid-IR pulses. The conditions for the excitation were identical for all samples so that signal magnitudes can be directly compared. In the depicted data set for T_LpT_L (Figure 1) all marker bands for the CPD formation can be traced back to ∼1 ps. For smaller delay times <1 ps the signal is obscured by nonlinear effects and the IR absorption of the initially formed excited state. With reference to the measurements on $(dT)_{18}$[10] the spectral changes after 1 ps can be assigned to vibrational cooling of newly formed CPD and the recovered T_LpT_L. This shows that also for T_LpT_L CPD

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forms during the decay (<1 ps)[7] of the $^1\pi\pi^*$ state. Common kinetic behavior of all samples can be inferred from time traces of the IR difference absorption. Traces at the spectroscopic location of one marker band (at 1404 cm^{-1}) feature a pronounced decay within less than 1 ps due to the decay of the $^1\pi\pi^*$ state and the disappearance of time zero artifacts (Figure 2). Vibrational cooling is manifest on a time scale of 10 ps. Thereafter the signals are essentially constant throughout 3 ns. The heights of these plateaus differ for the samples and increase with the dimerization yield φ_D^{cw}. Since these heights are a measure for the "early" dimerization yield φ_D^{ps}, this gives evidence for a clear correlation of the two yields.

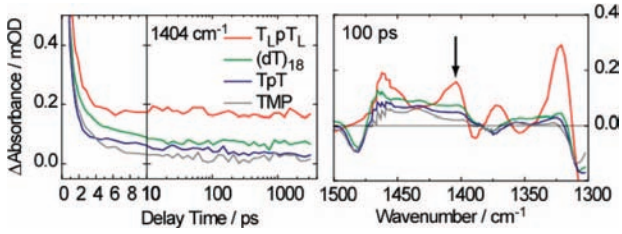

Figure 2. Comparison of the femtosecond IR difference signals for the three dimerizable thymine samples and the nondimerizable reference sample TMP. All signals are to scale. Left: Time trace for a detection wavenumber of 1404 cm^{-1}. Right: IR difference spectra recorded 100 ps after photoexcitation. Signal strengths appear in the order of the dimerization yields. The arrow marks the spectral location for the time traces on the left.

For a quantitative evaluation of the early CPD yield φ_D^{ps} measured after the decay of the $^1\pi\pi^*$ state, transient spectra were compared. The depicted spectra (Figure 2 right) were recorded 100 ps after photoexcitation. Note that 100 ps are long as compared to vibrational cooling times[20−23] but short in comparison to the 140 ps assigned to the triplet decay.[14] The diagram also includes the spectrum of the nondimerizable TMP. On the relevant time scale TMP only undergoes photophyiscal processes like internal conversion (IC) and intersystem crossing.[7] Since the dimerization yields of T_LpT_L, $(dT)_{18}$, and TpT are small (<15%), photophysical processes are also visible in these samples. The spectrum of TMP is used as a reference for these photophysical processes. The observed signal strengths of the dimerizable samples appear in the same order as the cw quantum yields φ_D^{cw}, i.e., $T_LpT_L > (dT)_{18} >$ TpT.

With knowledge of the corresponding extinction coefficients it is now possible to determine the picosecond quantum yields φ_D^{ps} (procedure given in the Supporting Information). The yields φ_D^{ps} were determined to be ∼1.5% (TpT), ∼3% ($(dT)_{18}$), and ∼10% (T_LpT_L). Considering the substantial error margins for both the cw and picosecond yields (±30%) the agreement between the two sets of yields is good. In particular, the ratios of the cw-values and the picosecond ones match. This gives very strong support that most of the CPD lesions are formed in an ultrafast process during the decay of the initially populated singlet state. The triplet path toward the CPD lesion does not show up under the nondiffusive conditions investigated here.

The observed ultrafast formation of the CPD lesion was the prerequisite for the hypothesis that the (small) fraction of reactive conformers holds responsible for the quantum yield. Reactive conformers are most likely those which feature base stacking. CD spectroscopy allows evaluation of base stacking properties of DNA.[24] Desnous et al.[17] have shown that TpT and T_LpT_L differ in CD activity by a factor of 3.7. The CD data recorded here (see Figure S3) reproduce the result and show further that the CD activity of $(dT)_{18}$ ranges between the other two. So the CD signatures of the three samples display the same ordering as the corresponding quantum yields.

Three samples with a yield for CPD formation ranging from 1.5% to 10% were investigated with cw and femtosecond IR spectroscopy. The results show that in all samples the absorption changes characteristic for CPD formation are present after ∼1 ps. The amount of CPD damage formed within picoseconds equals the amount recorded in steady-state experiments within the error margin. There are no absorption changes on the 100 ps to ns time scale which could be related to a delayed CPD formation. The observations are consistent with CPD formation via a singlet channel and rule out a significant contribution of a triplet pathway. The ultrafast CPD formation explains the correlation between structural indicators and dimerization yields. Therefore the propensity for CPD damage is controlled by DNA structure.

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Supporting Information Available: Reaction scheme for CPD formation, materials section, spectral assignment of CPD lesion, calculation of extinction coefficients, and CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Taylor, J. S. *Acc. Chem. Res.* **1994**, *27*, 76–82.
(2) Vink, A. A.; Roza, L. *J. Photochem. Photobiol. B: Biol.* **2001**, *65*, 101–104.
(3) Beukers, R.; Berends, W. *Biochim. Biophys. Acta* **1960**, *41*, 550–551.
(4) Beukers, R.; Eker, A. P. M.; Lohman, P. H. M. *DNA Repair* **2008**, *7*, 530–543.
(5) Johns, H. E.; Delbruck, M.; Rapaport, S. A. *J. Mol. Biol.* **1962**, *4*, 104–114.
(6) Whillans, D. W.; Johns, H. E. *J. Am. Chem. Soc.* **1971**, *93*, 1358–1362.
(7) Crespo-Hernandez, C. E.; Cohen, B.; Kohler, B. *Chem. Rev.* **2004**, *104*, 1977–2019.
(8) Gustavsson, T.; Banyasz, A.; Lazzarotto, E.; Markovitsi, D.; Scalmani, G.; Frisch, M. J.; Barone, V.; Improta, R. *J. Am. Chem. Soc.* **2006**, *128*, 607–619.
(9) Eisinger, J.; Shulman, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 895–900.
(10) Schreier, W. J.; Schrader, T. E.; Koller, F. O.; Gilch, P.; Crespo-Hernandez, C. E.; Swaminathan, V. N.; Carell, T.; Zinth, W.; Kohler, B. *Science* **2007**, *315*, 625–629.
(11) Martinez, J. M.; Elmroth, S. K. C.; Kloo, L. *J. Am. Chem. Soc.* **2001**, *123*, 12279–12289.
(12) Marguet, S.; Markovitsi, D. *J. Am. Chem. Soc.* **2005**, *127*, 5780–5781.
(13) Law, Y. K.; Azadi, J.; Crespo-Hernandez, C. E.; Olmon, E.; Kohler, B. *Biophys. J.* **2008**, *94*, 3590–3600.
(14) Kwok, W. M.; Ma, C.; Phillips, D. L. *J. Am. Chem. Soc.* **2008**, *130*, 5131–5139.
(15) Johns, H. E.; Pearson, M. L.; Helleiner, C. W.; Leblanc, J. C. *J. Mol. Biol.* **1964**, *9*, 503–524.
(16) Tramer, Z.; Wierzcho, Kl; Shugar, D. *Acta Biochim. Pol.* **1969**, *16*, 83–107.
(17) Desnous, C.; Babu, B. R.; McIriou, C.; Mayo, J. U. O.; Favre, A.; Wengel, J.; Clivio, P. *J. Am. Chem. Soc.* **2008**, *130*, 30–31.
(18) Schrader, T.; Sieg, A.; Koller, F.; Schreier, W.; An, Q.; Zinth, W.; Gilch, P. *Chem. Phys. Lett.* **2004**, *392*, 358–364.
(19) Callis, P. R. *Annu. Rev. Phys. Chem.* **1983**, *34*, 329–357.
(20) Elsaesser, T.; Kaiser, W. *Annu. Rev. Phys. Chem.* **1991**, *42*, 83–107.
(21) Owrutsky, J. C.; Raftery, D.; Hochstrasser, R. M. *Annu. Rev. Phys. Chem.* **1994**, *45*, 519–555.
(22) Pecourt, J. M. L.; Peon, J.; Kohler, B. *J. Am. Chem. Soc.* **2001**, *123*, 10370–10378.
(23) Kuimova, M. K.; Dyer, J.; George, M. W.; Grills, D. C.; Kelly, J. M.; Matousek, P.; Parker, A. W.; Sun, X. Z.; Towrie, M.; Whelan, A. M. *Chem. Commun.* **2005**, 1182–1184.
(24) Cantor, C. R.; Warshaw, M. M.; Shapiro, H. *Biopolymers* **1970**, *9*, 1059–1077.

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