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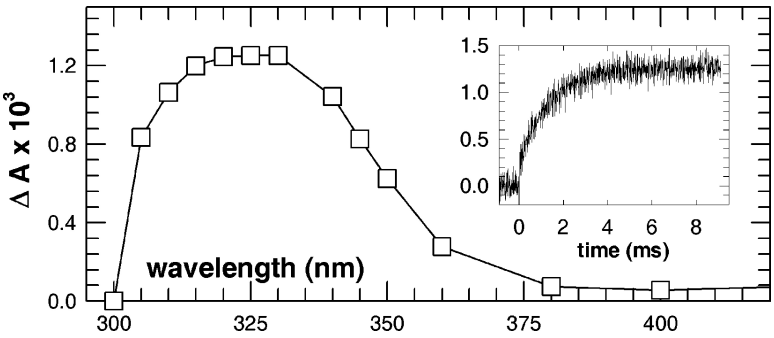

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Time-Resolved Study of Thymine Dimer Formation

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Absorption of UV radiation by DNA bases induces mutations which appear mainly at bipyrimidine sites.[1,2] The major photoproducts are cyclobutane dimers and pyrimidine-(6-4)-pyrimidone adducts. The latter are considered to result via oxetane or azedine intermediates which undergo ring opening.

Since the isolation of the first bipyrimidine dimers,[3−5] a large number of studies aimed at the understanding of the various factors which play a role in their formation have been published.[6] Despite the intense work in this field, there is still a complete lack of information regarding the time scales at which cyclobutane dimers and (6-4) adducts are formed. Laser flash photolysis experiments, which provide such information, are difficult to perform for DNA components. Indeed, data are distorted by excitation of accumulated photoproducts. Moreover, hydrated electrons and radical ions resulting from two-photon ionization[7] obscure absorption related to other processes occurring with low quantum yield. In the present laser flash photolysis investigation, dedicated to the single-stranded oligonucleotide, $(dT)_{20}$, we managed to overcome the above difficulties. We used continuous flow of the solutions and low excitation densities ($\leq$1.2 MW/cm^2) to avoid ionization of the studied systems. We show that direct excitation of the oligonucleotide leads to cyclobutane dimers (T<>T) in less than 200 ns, whereas the (6-4) adduct is formed within 4 ms via a reaction intermediate. A comparison between $(dT)_{20}$ and the corresponding mononucleotide (thymidine monophosphate, TMP) allows us to discuss the involvement of the triplet state in the dimer formation.

Our experiments were performed using the fourth harmonic (266 nm) of an Nd:YAG laser delivering 8 ns pulses at a repetition rate of 2 Hz. TMP and $(dT)_{20}$ were dissolved in phosphate buffer. The solutions were nitrogen purged. Measurements were carried out at room temperature. For both compounds, the optical density was adjusted to 0.24 (266 nm, 1 mm).

The transient spectra recorded for TMP solutions at 200 ns correspond to the well characterized TMP triplet,[8−10] peaking at 360 nm (Figure 2a). The transient signals decay at all wavelengths (280−600 nm) with the same time constant of 5 μs. The differential absorbance (ΔA) below 300 nm is negative because the molar extinction coefficient (ϵ) of the triplet is smaller than that of the ground state. When the same experiment is performed with the oligonucleotide, no transient absorption is detected between 300 and 700 nm (Figure 2a). Taking into account the sensitivity of our setup ($\Delta A = 2 \times 10^{-4}$), we conclude that, at 200 ns, the triplet concentration in the oligonucleotide solution is at least 1 order of magnitude lower than that of TMP.[11]

Despite the absence of any absorption band in the spectra recorded for $(dT)_{20}$ at the nanosecond and at the microsecond time scales, a band peaking at 325 nm, typical of the (6-4) adduct,[4,12] does appear at the millisecond time scale (Figure 2b). The transient signals recorded from 310 to 360 nm have the same time behavior. The (6-4) transient absorption reaches its maximum value within 4 ms (Figure 3a), and then it remains constant until, at least,

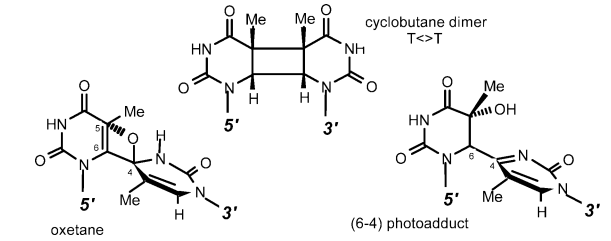

Figure 1. Structure of the dimeric thymine photoproducts.

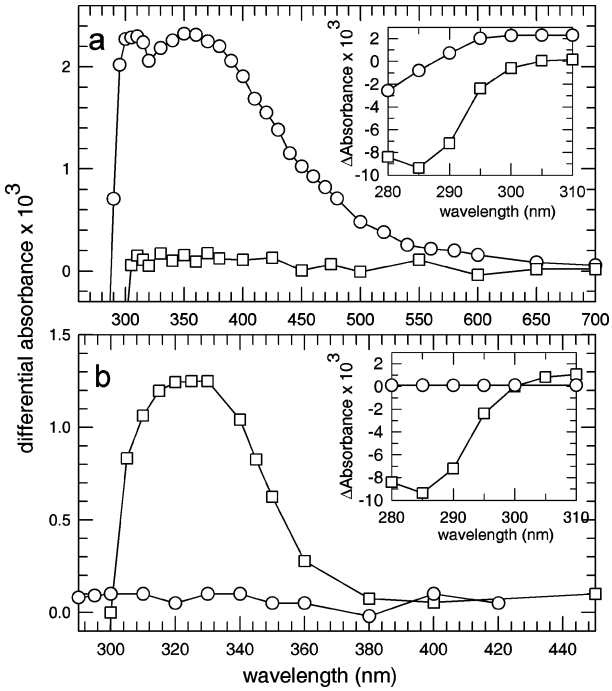

Figure 2. Transient absorption spectra recorded at 200 ns (a) and 10 ms (b) for TMP (○) and at $(dT)_{20}$ (□). Excitation density at 266 nm: 1.2 MW/cm^2.

100 ms. The same band is encountered in the steady-state absorption spectra of $(dT)_{20}$ solutions irradiated with 266 nm laser pulses (cf. Supporting Information). Excitation at this band gives the fluorescence spectrum of the (6-4) photoadduct[13] with a quantum yield of $(2.6 \pm 0.5) \times 10^{-2}$, in agreement with the literature value (3×10^{-2}).[12]

The delay with which the (6-4) absorption is observed proves that this adduct is formed via a reactive intermediate which absorbs below 300 nm. The oxetane (Figure 1), predicted to be a precursor of the (6-4) adduct,[5] is less conjugated than the (6-4) photoproduct and, therefore, is expected to absorb at lower wavelengths.

The signals obtained for $(dT)_{20}$ at the 280−300 nm range are negative and remain constant (Figure 2b) over the whole time domain probed (200 ns to 100 ms). This shows the formation of

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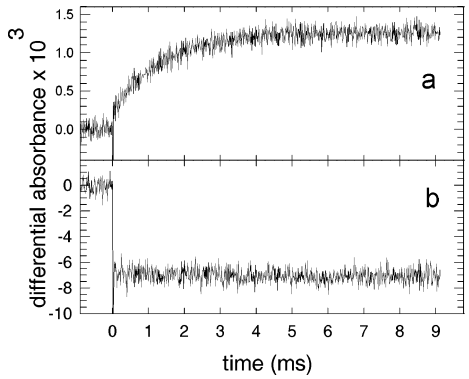

Figure 3. Transient signals obtained for $(dT)_{20}$ at 325 (a) and 290 nm (b). Excitation density at 266 nm: 1.2 MW/cm^2. None of the signals is affected when oxygen is bubbled in the solution.

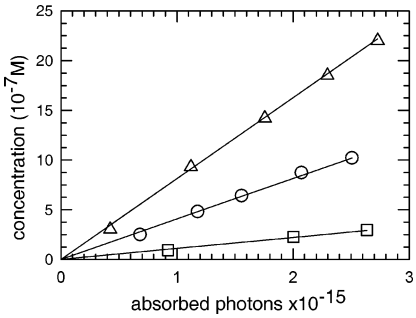

Figure 4. Concentrations of the photoinduced species as a function of the number of absorbed photons per pulse. TMP triplet at zero time (○) and (6-4) in $(dt)_{20}$ (□) are determined for ϵ_{max} values of 2300 M^{-1} cm^{-1},[10] and 4600 M^{-1} cm^{-1},[14] respectively. The T<>T concentrations in $(dt)_{20}$ (△) are calculated considering that the formation of one dimer results to the depletion of two thymine residues and that the ϵ of $(dT)_{20}$ at 290 nm is 1780 M^{-1} cm^{-1} per base.[15]

stable photoproducts in less than 200 ns. Since the signal intensity at 290 nm is not affected by the transformation of oxetane to (6-4), the contribution of these two compounds should be negligible. Therefore, the signal at 290 nm is attributed to the ground state depletion induced by the formation of T<>T dimers (four isomers).

The concentrations of the TMP triplet, as well as those of the dimers formed in the oligonucleotide, vary linearly with the number of absorbed photons. Consequently, the corresponding quantum yields can be determined from the slopes of the linear regressions in Figure 4. The intersystem crossing yield (ϕ_{ISC}) found for TMP is $(1.4 \pm 0.1) \times 10^{-2}$, in excellent agreement with that reported previously.[8] The yields associated to T<>T and (6-4) formation in $(dT)_{20}$ are $(2.8 \pm 0.2) \times 10^{-2}$ and $(3.7 \pm 0.3) \times 10^{-3}$, respectively. The quantum yield determined for the (6-4) formation from the steady-state absorption spectra of laser-irradiated solutions is $(3.5 \pm 0.4) \times 10^{-3}$, in agreement with that determined from the transient absorption measurements.

These values are 2−3 times higher than those derived from continuous irradiation of the dinucleoside TpT at 266 nm (1.3×10^{-2} for T<>T and 8×10^{-4} for (6-4)).[4] Such a difference can be explained by a lower dimerization probability in TpT as compared to that in $(dT)_{20}$ for which each thymine can react with either neighbor.

The absence of any triplet absorption from the transient spectra of $(dT)_{20}$ may be due to a decrease in ϕ_{ISC} upon oligomerization by at least 1 order of magnitude ($\phi_{ISC} < 1.4 \times 10^{-3}$). Such a change could arise from interactions between singlet excited states, revealed by fluorescence polarization measurements.[16] Another explanation is that the triplet state reacts in less than 200 ns with quasi unit efficiency to yield cyclobutane dimers since T<>T are known to be formed under triplet photosensitization conditions.[1] The data presently available do not allow us to favor one of the above hypotheses.

In conclusion, this investigation constitutes the first insight regarding the time scales at which the two major classes of pyrimidine dimers are formed. In particular, it brings experimental evidence that the (6-4) formation proceeds via a reaction intermediate. It opens new perspectives for the understanding of the dimerization mechanisms by examining the effect of biologically relevant factors, such as base sequence, base stacking, or base pairing.

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Supporting Information Available: Details on the experimental setup, experimental procedure, determination of the quantum yields, and steady-state spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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