## Supporting Information for

# Time-Resolved Study of Thymine Dimer Formation

Sylvie Marguet and Dimitra Markovitsi\*

Laboratoire Francis Perrin CEA/DSM/DRECAM/SPAM - CNRS URA 2453

CEA Saclav. 91191 Gif-sur-Yvette. France

Email : dimitra.markovitsi@cea.fr

#### Materials

The TMP nucleotide and the single stranded oligonucleotide  $(dT)_{20}$  were obtained from Sigma and Eurogentec Aldrich, respectively, and were used without further purification. The compounds were dissolved in phosphate buffer (pH 6.6), prepared using ultra pure water produced by a MILLIPORE purification system. Dihydrate quinine sulfate was obtained from Prolabo and was dissolved in HClO<sub>4</sub> 0.1 M..

#### Flash photolysis experimental details

Laser flash photolysis experiments were performed with a conventional apparatus. The Nd:YAG laser was a Spectra-Physics Quanta Ray model. The repetition rate was 2 Hz. The probing light (450 W Xenon arc) was observed at right angle with respect to exciting beam, dispersed in a Spex monochromator, detected by a Hamamatsu R938T photomultiplier and analyzed by a Tektronix DSA 620 oscilloscope. Transient absorption spectra were recorded using a point-by-point approach. Transient signals were averaged over 50 - 300 shots. The transient signals were averaged over 50 and 300 laser. The lamp stability allowed us to record transient signals up to 100 ms with a sensitivity of  $2 \times 10^{-4}$  in differential optical density (Figure 1).



Figure 1: Fluctuation of the Xenon arc lamp at 100 ms time scale at 325 nm. Average over 100 shots

All measurements were carried out in nitrogen-purged buffer at room temperature. About 60 ml of solutions were kept flowing in a 1mm x 10mm quartz cell. The flow was adjusted in such a way that each laser pulse excites a fresh volume of solution. The transit time of the irradiated solution in front of the probing light is of the order of 100 ms which determines the upper limit of the probed time domain. Fast shutters were placed in the path of both laser and lamp beams in order to minimize sample exposure to both light sources.

The energy was measured by an energy ratiometer (Laser precision Instruments, Rj 7200). The laser energies at 266 nm were attenuated to  $\leq$  5.4 mJ with a half-wave plate corresponding to a maximum excitation density of 1.20 MW/cm<sup>2</sup>. Under these conditions, no hydrated electron  $(e_{aq})$  coming from ionization of the buffer or the studied compounds was detected ( $[e_{aq}] < 2x10^{-8}$  M). For both compounds, the optical density at 266 nm were adjusted to 0.244 ± 0.002 (266 nm, 1 mm). The absorbed energies were calculated from the difference of two measurements: the transmitted energy behind a cell containing either the buffer alone or the compound dissolved in the buffer. For an excitation density of 1.20 MW/cm<sup>2</sup>, the energy absorbed by the two compounds was 1.84 mJ, *i.e.* 2.46x10<sup>15</sup> photons/pulse. This corresponds to a equivalent concentration of photons [ph] equal to 7.3x10<sup>-5</sup> einstein/l, obtained from the following equation:

(1)

 $10^{-3}$  [ph] S l N<sub>av</sub> = I<sub>abs</sub> with I<sub>abs</sub> = 2.46 10 <sup>15</sup> photons/pulse, S = 0.56cm<sup>2</sup>; l = 0.1 cm; N<sub>av</sub> = 6.02 10<sup>23</sup> S is the irradiated surface and I the optical pathway along the laser beam direction.

## Determination of the concentrations of the triplet state of TMP

For TMP, the concentration of the triplet state as a function of time  $([T_1]_{(t)})$  is related to the differential absorbance  $(\Delta A_{(t, \lambda)})$  according to the general equation :

(2) 
$$\Delta \mathbf{A}_{(t,\lambda)} = (\varepsilon_{(\lambda)}^{T_1} - \varepsilon_{(\lambda)}^0) [T_1]_{(t)}$$

At 360 nm, there is no absorption of the ground state and only the triplet state absorbs. The concentration of the triplet state at zero time  $[T_1]_{(t=0)}$  is determined from the zero time of the differential absorbance  $\Delta A_{(t=0,360nm)}$  and the molar extinction coefficient determined by Gut et al.,  $(\epsilon_{(360)})^{T1} = 2300 \text{ M}^{-1} \text{ cm}^{-1}$ .<sup>[1]</sup>

## Determination of the concentrations of the two photoproducts of (dT)<sub>20</sub>

In the case of  $(dT)_{20}$ , the differential absorbance as a function of time and wavelength is given by

(3)  $\Delta A_{(t,\lambda)} = (\epsilon_{(\lambda)}^{cyclo} - 2\epsilon_{(\lambda)}^{0}) [cyclo]_{(t)} + (\epsilon_{(\lambda)}^{6-4} - 2\epsilon_{(\lambda)}^{0}) [(6-4)]_{(t)} + (\epsilon_{(\lambda)}^{oxe} - 2\epsilon_{(\lambda)}^{0}) [(oxe)]_{(t)}$ 

where  $[cyclo]_{(t)}$ ,  $[(6-4)]_{(t)}$  and  $[oxe]_{(t)}$  denote the concentration of cycobutane dimers, (6-4) adduct and oxetane, respectively;  $\varepsilon_{(\lambda)}^{cyclo}$ ,  $\varepsilon_{(\lambda)}^{6-4}$  and  $\varepsilon_{(\lambda)}^{oxe}$  denote the molar extinction coefficients of these photoproducts.

#### Determination of the final (6-4) concentration

At 325 nm, only the (6-4) adduct absorbs and the previous relation is reduced to:

(4) 
$$\Delta A_{(t,325nm)} = (\epsilon_{(325)}^{6-4}) [(6-4)]_{(t)}$$

The concentration of (6-4) at 10 ms is determined from  $\Delta A_{(t=10ms,325nm)}$  and eq. 4, and the molar extinction coefficient determined by Douki et al., ( $\epsilon_{(325)}^{6-4} = 4600 \text{ M}^{-1} \text{ cm}^{-1}$ ).<sup>[2]</sup>

## Determination of cyclobutane dimers concentration

The cyclobutane dimers does not absorb at 290 nm:

(5) 
$$\Delta A_{(t,290\text{nm})} = (-2 \varepsilon_{(290)}^{0}) [\text{cyclo}]_{(t)} + (\varepsilon_{(290)}^{6-4} - 2 \varepsilon_{(290)}^{0}) [(6-4)]_{(t)} + (\varepsilon_{(290)}^{0} - 2 \varepsilon_{(290)}^{0}) [(\text{oxe})]_{(t)}$$

Since the signal intensity at 290 nm is not affected by the transformation of oxetane to (6-4), we conclude that the contribution of these two compounds should be negligible. Thus, equation (5) is can be approximated by :

(6)  $\Delta A_{(t,290nm)} = (-2 \epsilon_{(290nm)}^{0}) [cyclo]_{(t)}$ 

The concentration of cyclobutane dimers is determined from  $\Delta A_{(t,290nm)}$  and eq. 6, and the molar extinction coefficient of the ground state of  $(dT)_{20}$  determined by .Riley et al.,  $(\epsilon_{(290)})^0 = 1780 \text{ M}^{-1} \text{ cm}^{-1}$ .<sup>[3]</sup>

### Determination of the quantum yield $\Phi_x$

The quantum yield of photoinduced species corresponds to the number of species formed per number of absorbed photons and can be calculated from the ratio of two concentrations:

 $\Phi_x = [x]/[ph]$  with "x" denotes the transient species and "ph" the absorbed photons.

The quantum yields are thus obtained from the slopes of the linear regressions in Figure 4, considering that  $2.46 \times 10^{15}$  absorbed photons/pulse correspond to [ph] =  $7.3 \times 10^{-5}$  einstein/l.

#### Steady-state absorption and fluorescence measurements of laser irradiated solutions

Absorption spectra were recorded with a Perkin Lamda 900 spectrophotometer. Fluorescence spectra were recorded by means of a SPEX Fluorolog-2 spectrofluorometer with a bandpass of 4.71 nm at excitation side and 2.25 nm at the emission side. The light source was a 450 Watt arc Xenon lamp. The calibration of emission monochromator was verified using a Hg low pressure standard lamp. The fluorescence spectra were corrected for the instrument response.

We have recorded the steady state absorption spectra of  $(dT)_{20}$  solutions at the 290 - 500 nm region before and after irradiation with a 267 nm pulses. The difference between these two spectra exhibits a band peaking at 325 nm, showing that the species detected in the transient absorption measurements corresponds to a stable photoproduct. The concentration determined from the steady-state optical density for a given number of absorbed photons per volume allows the determination of the quantum yield for the (6-4) formation:  $(3.5 \pm 0.4) \times 10^{-3}$ .



Figure 2: Steady-state differential absorption spectrum of a  $(dT)_{20}$  solution irradiated with 267 nm laser pulses (1.2 MW/cm<sup>2</sup>; total number of absorbed photons per liter:  $6.0 \times 10^{-4}$  einstein/l; optical path-length: 10 mm)

Excitation of the irradiated solutions at wavelengths 310 - 340 nm gives fluorescence spectra peaking at 406 nm (Figure 3).



Figure 3: Steady-state fluorescence spectrum of a  $(dT)_{20}$  solution irradiated with 267 nm laser pulses;  $\lambda_{exc} = 315$  nm

#### REFERENCES

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