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UV-Induced TA Photoproducts: Formation and Hydrolysis in Double-Stranded DNA

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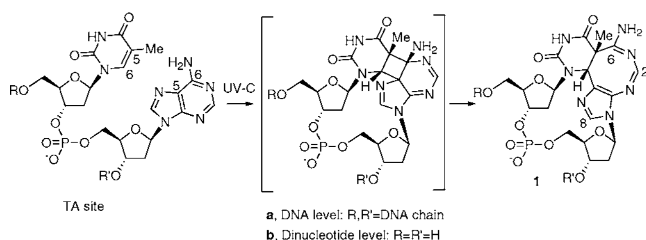
Abstract: Hydrolysis of TA photoproduct leads to two derivatives presenting different formation kinetic profiles depending on the oligomer content. The formation efficiency of TA photoproducts in UV-C-irradiated DNA slightly exceeds the formation of the *trans*,*syn* cyclobutane pyrimidine dimer at TT sites.

Dipyrimidine sites in DNA are well recognized to be the major targets of photochemical damage resulting in the production of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts ((6-4) PPs).[1] In humans, these photoproducts are involved in solar-induced skin cancer and photoaging.[2] Pyrimidine-purine sites are also photoreactive.[3,4] The TA site gives rise to photoproduct **1a** that is also produced by spontaneous deamination of the photoproduct formed between 5-methylcytosine (m^5C) and adenine. This largely unstudied TA photoproduct has recently been shown to possess important mutagenic properties.[5] In addition, it might also interfere with gene regulation through its formation in the TATA promoter sequence.[3a,5a,6] The initial photochemical event occurring at TA sites is not yet fully elucidated.[7] It is assumed to involve a [2+2] cycloaddition reaction between the C5−C6 double bond of the 5′-thyminyl residue and the C6 and C5 positions of the 3′-adenyl residue (Scheme 1). The resulting cyclobutane adduct, which was originally proposed as the TA photoproduct,[8] actually fragments to yield the more stable 1,3-diazacyclooctatriene derivative **1**,[9] whose structure was confirmed by X-ray crystallography at the dinucleotide level (Scheme 1).[10]

Scheme 1



The biological relevance of **1a** depends on several factors. One is its frequency of occurrence. So far, the formation efficiency of **1a** has only been estimated by fluorescence or radiochemical measurement of 6-methylimidazo[4,5-*b*]pyridin-5-one (6-MIP), its acid hydrolysis product.[3] An estimated correction factor was applied to take into account the efficiency of transformation of **1b** into 6-MIP,[3b] even though 6-MIP formation at the dinucleotide level and that in the DNA context are not necessarily similar. Another parameter that might have hampered the accurate quantification of **1a** is its instability.[5a,11] Indeed, in aqueous solution at room temperature **1a** affords a "hydration" product[12] (**2a**) of unknown biological importance. It has been hypothesized that, in water, the C2 or C6 amidine functionality of **1a** would hydrolyze to its corresponding hemiaminal or formamide derivative.[12] Hitherto, however, the chemical structure of **2a** has remained unresolved.

All these uncertainties are a disadvantage since accurate identification and quantification of individual primary and secondary PPs in DNA are critical to fully address their contribution to the biological effect of UV light.

Herein, we unambiguously elucidate the structure **2** at the dinucleotide level and quantify its formation along with that of **1** in UV-irradiated DNA. In addition, another TA hydrolysis PP is reported whose formation is only observed in DNA.

Upon standing at room temperature in D_2O, **1b** was partially converted into **2b**, as evidenced by ^1H NMR spectroscopy (see Supporting Information (SI) Figure S3). These two compounds were separated by RP-HPLC. High-resolution mass spectrometry data of **2b** (ESI, $(M - H)^-$ cald for $C_{20}H_{27}N_7O_{11}P$ 572.1506, found 572.1516) confirmed the addition of H_2O with respect to **1b**. The major difference observed on the ^1H NMR spectrum of **2b** recorded in D_2O with respect to that of **1b** was the deshielding (δ 8.33) of one of the unsaturated proton singlets (δ_{H2} 7.28; δ_{H8} 7.83 in **1b**[8b]). Carbon C8 of **2b** (δ 136.3) was unambiguously assigned by its correlation with H1′ of the 2′-deoxyadenosinyl residue (-pdA) (^3J) on the HMBC spectrum. Therefore, H8 was easily attributed to the signal at δ 7.92 (HSQC correlation), confirmed by the NOE between H8 and H2′ of -pdA. Therefore, the deshielded proton at 8.33 ppm was attributed to H2. C2 in **2b** (HSQC correlation) was deshielded compared to that in **1b** (δ_{C2} 144.2[9]). It appeared at δ 168.4, a typical value for a N-formyl group.[13] H2 correlated with one quaternary carbon of the imidazole moiety (δ 123.2) on the HMBC spectrum, indicating that the formyl group was necessarily located on the ^3N atom. Consequently, the formamide structure **2b** was attributed to the hydrolysis product of the TA PP. Interestingly, **2b** also gives rise to 6-MIP upon acid hydrolysis (see SI pp S-9−S-13).

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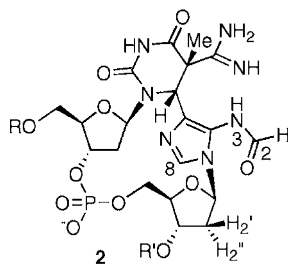

a, DNA level: R,R'=DNA chain
b, Dinucleotide level: R=R'=H

We have previously reported an accurate method for the quantification of dipyrimidine PPs in UV-irradiated DNA.[14] This determination relies on a combination of enzymatic release of the PPs as dinucleoside monophosphates, HPLC separation of each dinucleotide PP, and tandem mass spectrometry quantification. For accurate and quantitative analyses, calibrated solutions of standards are required for each PP. With compounds **1b** and **2b** in hand, we extended the application of our method to the identification and quantification of **1** and **2** in UV-C-irradiated calf thymus DNA, given that **1b** and **2b** were stable to the enzymatic hydrolysis conditions (SI Figure S14). To allow a precise comparison with known PPs formation efficiency, the formation of PPs at TT sites was also simultaneously monitored.

Photoproduct **1b** was readily detected on the HPLC-MS chromatogram of UV-C- and -B-irradiated DNA (SI Figures S17 and S18). In contrast, **2b** was not detected immediately after irradiation but was unambiguously observed when irradiated DNA was incubated at 37 °C for increasing periods of time prior to enzymatic digestion (Figure 1).

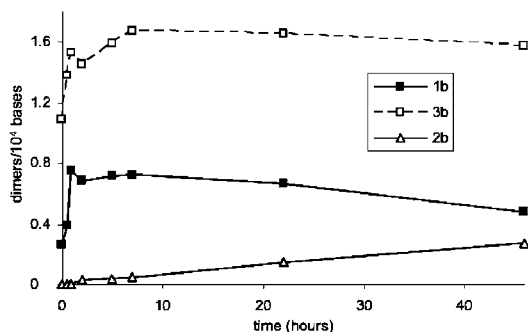

Figure 1. Yield of TA photoproducts in UV-C-exposed DNA incubated at 37 °C for increasing periods of time.

Interestingly, the yield of **1b** significantly increases in the first hour of incubation, a process possibly related to the conversion of the initial cyclobutane adduct. More importantly, a compound (**3b**) that elutes close to **2b** and exhibits similar mass spectrometric features was also detected as an additional immediate irradiation product (Figures 1 and S17). Compound **3b** was not detected when TpdA was exposed to UV-C radiation (SI Figure S19). We thus propose that **3b** is a hydrolysis product of a TA PP favored in double-stranded (ds) DNA. It may derive from the initial cyclobutane adduct, as suggested by its increased formation in the early times of incubation of irradiated DNA.

Linear regression of the dose−course plot for formation of PPs (SI Figure S20) was used to calculate the quantum yield (φ) of TT and TA PPs per TT and TA sites in DNA, respectively. Formation of TT PPs was in accordance with previous results.[14a] As anticipated, *cis,syn* T(CPD)T and T(6-4)T were the major TT photoproducts within UV-C-irradiated calf thymus DNA ($\varphi = (18 \pm 3) \times 10^{-3}$ and $(1.6 \pm 0.3) \times 10^{-3}$, respectively).[14a] The formation efficiency of **1** ($\varphi = (3 \pm 0.4) \times 10^{-5}$) was lower than that of *trans,syn* T(CPD)T ($\varphi = (2 \pm 0.3) \times 10^{-4}$). Importantly, the yield of **3** ($\varphi = (3 \pm 0.5) \times 10^{-4}$) was higher than those of **1** and *t,s* T(CPD)T (Figure 1). The cumulative yield of **1a** and **3a** represented 1.8×10^{-2} times the formation yield of *c,s* T(CPD)T. The originally reported formation efficiency of TA photoproduct per TA site in calf thymus DNA[3b] ($\varphi = 10^{-4}$) was fortuitously close to our result, despite several approximations.

In conclusion, we have unambiguously characterized the hydrolysis product of **1b** and shown that this slow process can also occur in DNA. More importantly, we provide evidence for the formation of another TA hydrolysis photoproduct solely in ds DNA, although additional work is necessary to establish its structure. Finally, TA PPs formation is a process whose efficiency slightly exceeds that of *t,s* T(CPD)T.

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Supporting Information Available: Experimental conditions, NMR spectra, HPLC chromatograms, and dose−course formation of PPs. This material is available free of charge via the Internet at http://pubs.acs.org.

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