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DNA Photochemistry: Geometrically Unconstrained Pyrimidine (6−4) Pyrimidone Photoproducts Do Photoisomerize

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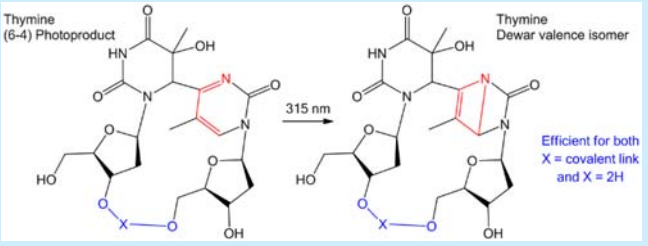
Supporting Information

ABSTRACT: Structural features are of major importance for the formation of mutagenic photoproducts in DNA. It was recently reported that lack of constraints between two adjacent nucleosidic units prevents the conversion of pyrimidine (6−4) pyrimidone photoproducts into their Dewar valence isomers. We here report that this is not the case for the thymidine photoproducts which, although unconstrained, are quantitatively converted into photolysis products identified as Dewar valence isomers by mass spectrometry and NMR and infrared spectroscopies.



Solar light exhibits carcinogenic properties which are explained by the absorption of UV photons by DNA bases that triggers dimerization photoreactions between adjacent thymines and/or cytosines. Pyrimidine(6−4)-pyrimidone photoproducts (64PPs) and cyclobutane pyrimidine dimers (CPDs) are the major classes of DNA lesions induced through this pathway. Major efforts are made in photochemistry to link their formation with the initially produced excited states.[1−7] From a chemical point of view, 64PPs are unique among DNA lesions due to the presence of the pyrimidone ring which exhibits a maximum of absorption in the UVB range associated with fluorescence emission. In addition, the pyrimidone moiety of 64PPs can undergo photoisomerization into the related Dewar valence isomers (Dewars) (Figure 1), as characterized in dinucleoside monophosphates.[8−12] Dewars are biologically relevant lesions since they are detected in cells and skin exposed to natural and simulated solar radiation.[13−18] Recently, formation of Dewar was investigated by time-resolved IR spectroscopy in dinucleotidic probes where the phosphodiester bond was replaced by either a silyl or a formacetal linker,[19,20] and a relatively low reaction rate could be determined. A surprising observation in this study was that a 64PP where the link between the nucleosidic units was cleaved was no longer photoconverted into its Dewar isomer. A theoretical approach explained this result by a requirement of geometrical constraints for the photoconversion of 64PPs into Dewars.[21] This major role of structural features is reminiscent of the ultrafast formation of CPDs which takes place only at dinucleotides exhibiting a favorable orientation.[22]

However, the inhibition of Dewar formation in the absence of internucleosidic link is not in agreement with other observations related to photoproducts of monomeric model systems lacking covalent bonding between the deoxyribose units. First, Varghese reported as early as in 1971 that the 64PP of thymidine (Thd) was lost upon exposure to UVB radiation.[23] More recently, we reported that the thymine and Thd 64PPs were quantitatively converted into dimeric photoproducts[24] tentatively identified as the corresponding Dewars, although no extensive characterization was undertaken. These contradictory observations prompted us to perform a more thorough study of the photolysis of Thd 64PP in order to determine whether or not Dewars were produced. We first unambiguously confirmed the quantitative photoconversion of

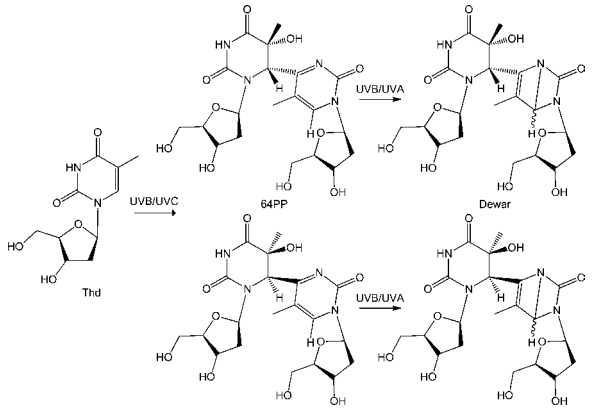


Figure 1. Formation of 64PPs of thymidine and their photoisomerization into Dewars. Two diastereoisomers of 64PPs are isolated. Each of them then gives rise to 2 Dewars bearing an asymmetric carbon 6 on the pyrimidone ring.

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Thd 64PPs upon exposure to UVB, using a lamp emitting a broad spectrum ranging from 280 to 370 nm with a maximum at 312 (see the Supporting Information). Solutions of the two diastereoisomeric 64PP 1 and 64PP 2 of Thd were prepared as previously reported[24] and exposed to UVB for increasing periods of time up to 2 h. On the basis of the area of the peaks recorded during HPLC analysis with UV detection set at 315 nm (Figure S1, Supporting Information), we observed the consumption of 64PP 1 and 64PP 2 (Figure 2). The conversion

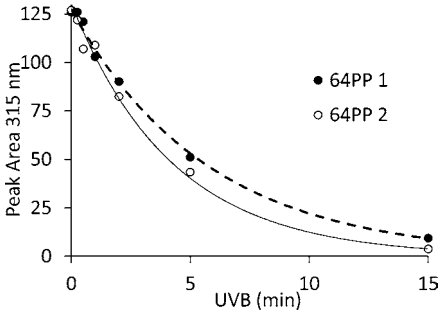

Figure 2. Photolysis of the two diastereoisomers of 64PPs of Thd upon exposure to UVB radiation. Solutions (15 μM) were exposed to UVB under stirring and analyzed by HPLC with a UV detector set at 315 nm. The reported results are the area of the integrated HPLC peaks and are fitted with a single exponential.

yield was larger than 90% yield after 15 min and 99% after 2h. The photolysis products of 64PPs completely lacked 315 nm absorption as shown by the absence of peaks on the chromatogram after 2 h of exposure to UVB (Figure S1, Supporting Information).

The photolyzed samples were further analyzed by HPLC interfaced with an electrospray triple quadrupolar mass spectrometer recording the fragmentation mass spectra of compounds exhibiting the molecular weight of Thd dimers (pseudomolecular ion M + H^+ at m/z = 485). Following 2 h exposure to UVB, 64PPs were no longer detected by HPLC–MS/MS, and two new peaks were observed on the chromatograms for each of the photolyzed 64PP (Figure 3). These photolysis products were referred to as Dewar 1-1 and 1-2 for the photolysis products of 64PP 1 and Dewar 2-1 and 2-2 for 64PP 2. In the photolyzed solution of 64PP 1, one of the new compounds had the same retention time as the initial 64PP but lacked the 315 nm absorption and exhibited different mass fragmentation properties. All Dewars exhibited a fragmentation mass spectrum richer than their 64PP precursors (Figure 4, Figures S2 and S3 (Supporting Information), and Table 1). This result may be related to the fact that Dewars are less chemically stable than 64PPs.[25,26] The observation of a pseudomolecular ions at m/z = 485 and the presence of ions corresponding to the loss of one deoxyribose unit (m/z = 369) and another to two covalently linked thymine bases (m/z = 253) confirmed that the four identified photolysis products of 64PPs were thymidine dimeric photoproducts. In addition, rationalization of the other observed fragmentations suggested that they actually were Dewar valence isomers (Figure S4, Supporting Information). Using a mass range where Dewars produced much more daughter ions than 64PPs, namely between m/z = 110 and 230, we then determined the time-course formation of the four photoisomers by HPLC–MS/MS. We found that it was concomitant with the loss of the 64PPs and that Dewars were stable upon irradiation for 2 h (Figure

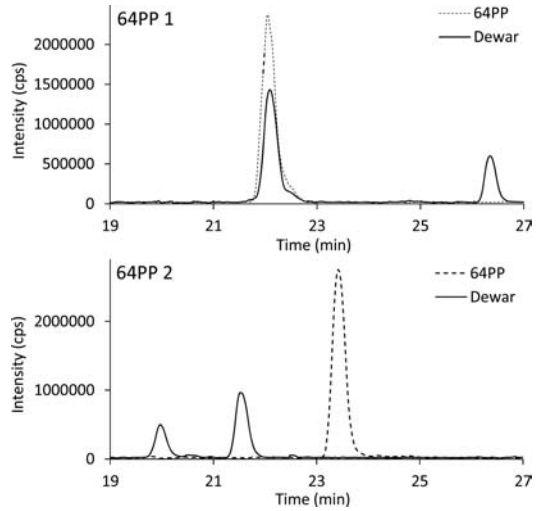

Figure 3. HPLC–MS/MS chromatograms recorded before and after a 120 min UVB irradiation of Thd 64PP 1 and 64PP 2. The mass spectrometer was used in the daughter ion mode, with the pseudomolecular ion set at m/z = 485 and the daughter ions analyzed over the 100–500 range.

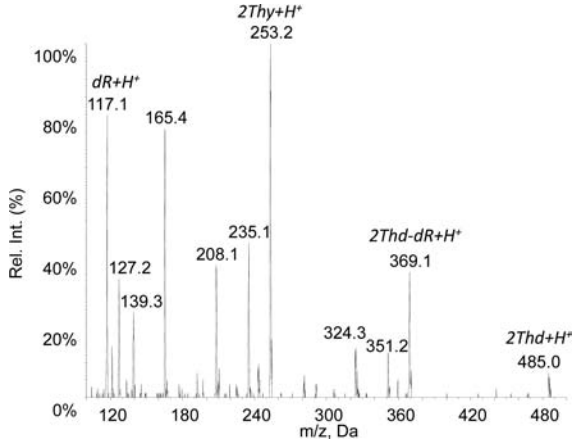

Figure 4. Fragmentation mass spectra of Dewar 2-1 recorded upon HPLC–MS/MS analysis of a solution of pure 64PP 2 exposed to UVB radiation. Thd: thymidine, Thy: thymine, dR: dehydrated 2-deoxyribose.

Table 1. Mass Fragmentation Characterization of the 64PPs and Dewars of Thd[a]

	64PP 1	64PP 2	Dewar 1-1	Dewar 1-2	Dewar 2-1	Dewar 2-2
117	1	0	71	58	84	53
127	2	0	100	86	31	79
139	0	0	3	5	28	3
165	0	0	8	5	82	9
208	0	0	1	7	41	6
235	100	100	82	100	46	100
243	0	0	26	33	9	22
253	6	32	14	7	100	12
351	7	8	43	53	15	13
369	1	2	10	13	36	15

[a]Spectra were recorded online upon HPLC–MS/MS analysis. Spectra were normalized to the largest peak, and data are expressed in percent of this value. Only values for the most intense ions are reported.

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S5, Supporting Information). These observations allowed us to conclude that the discussed dimeric photolysis products of 64PPs were primary photoproducts, as expected for Dewar valence isomers.

In order to unambiguously characterize the 64PP photolysis products we then used ^{1}H NMR spectroscopy. For this purpose, we exposed a saturated solution of thymidine first to the 254 nm light emitted by a UVC in order to produce 64PPs. The solution was then exposed to UVB in order to convert 64PPs into Dewars. The latter photoproducts were then isolated by HPLC in amounts large enough to permit the recording of their ^{1}H NMR spectrum (Figure S6 and Table S1, Supporting Information). We observed the presence of signals corresponding to the deoxyribose units, and to the methyl and H6 protons of thymine moieties (Table 2). The ^{1}H NMR features of the aglycone of the Dewars are very similar to those of TpT Dewar.[9] The most significant information was that the 3′-end H6 is upfield shifted in Dewars (5.4 ppm) compared to that of 64PPs which resonates at approximately 8 ppm both in TpT[27] and thymine 64PPs.[28] We observed a similar trend for the methyl group of the pyrimidone ring which resonates around 2.3 ppm in 64PPs[27,28] and is upfield shifted below 2 ppm in Thd Dewars. These characteristics result from the conversion of the C6 carbon from a sp^2 hybridization in 64PPs to a sp^3 hybridization in Dewars and the loss of aromaticity of the pyrimidone ring in the latter photoproducts. It should be added that the signals for the H6 and methyl group of the pyrimidine moiety are similar in Dewars and 64PPs. To confirm the NRM data showing that the 64PP photolysis products are indeed Dewars, we recorded an infrared spectrum. We unambiguously observed the three bands at 1630, 1700, and 1780 cm^{-1} (Figure S7, Supporting Information) previously observed for Dewar isomers of other models.[8,19] The latter signal is particularly interesting since it was found to be present in the IR spectrum of Dewars but not of 64PPs.[8,19]

Table 2. Chemical Shifts of the Hydrogen Atoms of the Pyrimidone (Pyo) and Pyrimidine (Pyr) Moieties of Dewars[a]

photoproduct	H6 Pyo	H6 Pyr	CH_3 Pyo	CH_3 Pyr
Dewar 1-1	5.41	5.18	1.88	1.54
Dewar 1-2	5.42	5.11	1.87	1.55
Dewar 2-1	5.41	5.31	1.83	1.53
Dewar 2-2	5.43	5.20	1.86	1.53

[a]Spectra were recorded in D_2O at 400 MHz. Results are expressed in chemical shifts (ppm) with respect to HDO set at 4.79 ppm.

Altogether, our results confirm that 64PPs are converted into Dewars even in the absence of structural constraints. These results contrast with those recently reported and rationalized by theoretical calculations.[19−21] One explanation could be a wavelength issue. Indeed, a 10 nm blue shift of the maximal absorption is observed when comparing 64PP of TpT[27] with 64PP of thymine or Thd.[23,29] Experiments resulting in the lack of formation of Dewars[19] involved either white light, likely using a lamp containing UVA but limited UVB radiation, for continuous irradiation or 323 nm pulses for the time-resolved studies and were thus less favorable to absorption by unconstrained 64PPs. Second, it cannot be ruled out that of the absence of covalent bonds between the two bases in 64PPs leads to a decrease in the quantum yield of photoisomerization as it does for fluorescence.[30] This would be partly in line with the theoretical investigation showing that a lack of covalent link between nucleosidic units prevents photoisomerization in 64PPs,[21] with the slight difference that unconstrained 64PPs are flexible and may adopt conformations which make photoisomerization possible. Altogether, a lack of constraints may possibly decrease the quantum yield of photoconversion but not completely inhibit it. It should finally be stressed that structural features exhibit a strong impact on one aspect of the Dewar formation: the stereospecificity. Indeed, two diastereoisomeric Dewars are produced for each 64PP of Thd with the 3′-end C6 as an asymmetric carbon. This contrasts with the situation in dinucleoside monophosphates where only one diastereoisomer of Dewar is produced whatever the bases involved.[8,10,12] This observation shows that although structural features should not be systematically put forward to explain unexpected results they are very important parameters in DNA photochemistry.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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