(6-4) Photoproduct DNA photolyase mechanistic studies using s^5-(6-4) photoproducts

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The facile photoreversal reaction, which occurs upon direct irradiation of the thioanalogue of the (6-4) photoproducts of DNA 3, and not from its corresponding Dewar derivative 4, to give the parent dinucleotide 1 models (6-4) photoproduct DNA photolyase repair activity.

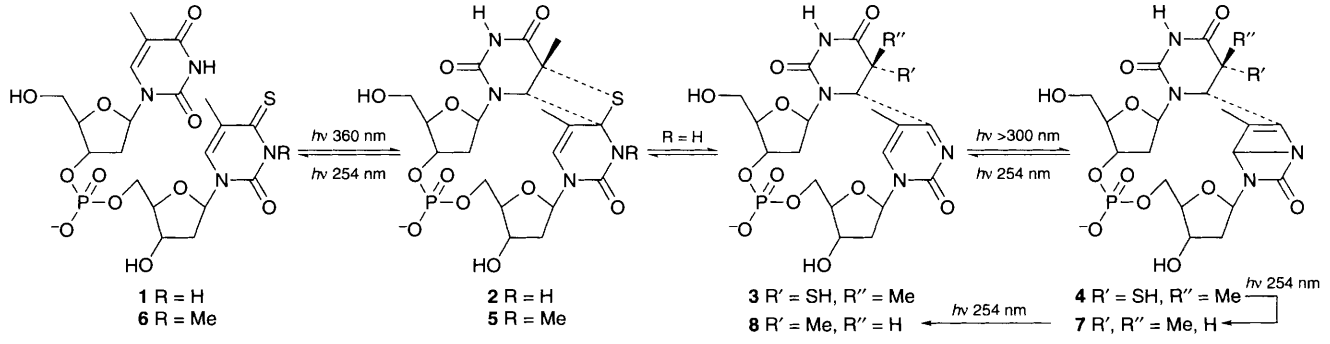
In human health, as a consequence of the ongoing stratospheric ozone depletion, it is anticipated that enhanced exposure to the UV portion of sunlight will provoke a considerable increase in the formation of highly mutagenic and carcinogenic types of DNA photoproducts, such as the cyclobutane pyrimidine photoproducts (CPDs) and (6-4) pyrimidine pyrimidinone photoproducts [(6-4)PPs], together with the Dewar valence isomers of the latter.[1,2] Although less readily formed than CPDs, (6-4)PPs might actually be more effective at causing damaging mutations.[3] Fortunately, the mutagenic potential of these adducts can be sizeably reduced by cellular enzymatic repair processes. Thus, the well studied DNA photolyase reverses specifically, through the action of visible light, the dimerized pyrimidines of CPDs *via* cyclobutane splitting.[4] A more ubiquitous process, operating on these types of DNA photoadducts, is nucleotide excision repair.[5] Recently, an additonal and original repair pathway, specific for (6-4) photoproducts, has been discovered consecutively to the isolation of new photoreactivating enzymes.[6] The enzymatic mechanism of this other type of photorepair is still unknown. Kim *et al.* have proposed two general pathways to account for the mechanism of the (6-4)PP photolyase.[7] The enzyme might either photochemically catalyse the formation of an oxetane (or azetidine) and thermally catalyse its cycloreversion to two thymines (or thymine and cytosine) or *vice versa*. The photochemical step would occur either after direct excitation, energy or electron transfer. Interestingly, in a mechanistic study related to the (6-4) photolyase mechanism, Prakash and Falvey[8] have thoroughly described the efficient photosensitized splitting reaction of an oxetane model under electron transfer conditions.

Some years ago, we observed that near UV irradiation of 5′-*O*-thymidylyl-4-thiothymidine (Tps^4T) **1** led to the formation of four photoproducts.[9] The important photoproducts of this reaction were thietane **2**, found in equilibrium with its opened form **3**, and the s^5-Dewar photoproduct **4**. It is evident that the mechanism of their formation parallels the reaction pathways leading to (6-4) photoproducts and that the photoproducts derived from **1** could serve in model reactions aimed at understanding the photo-enzymatic repair mechanism of (6-4) photoproducts by (6-4) photoproduct DNA photolyase.

In this context, we herein report that short wavelength irradiation† (254 nm) of a neutral aqueous solution containing an interconverting mixture of thietane **2** and (6-4) photoproduct **3** (3 : 1 ratio)[9] quantitatively restored **1** (Scheme 1). Under higher pH conditions (pH 10), which completely shifts the **2** ⟷ **3** equilibrium toward **3**, the reversal reaction did not occur. This clearly suggested that for the photoreversion to take place, the preliminary formation of the thietane intermediate **2** is absolutely required. This interpretation of the reaction mechanism was confirmed by irradiating the *N*-methyl thietane **5**,[10] since a quantitative photoconversion of **5** into the dinucleotide **6** was achieved under neutral and basic (pH 10) conditions, at roughly the same rate as in the case of the **2** ⟷ **3** equilibrium.

These results were obtained by HPLC and NMR spectroscopic analysis which showed the photoreversion of **2** ⟷ **3** and **5** at pH ≃ 7 and **5** at pH 10 to be nearly complete within 10 min under our conditions. Inspection of the UV ($\lambda_{max.}$ ≃ 265 and 335 nm) and ^1H NMR spectra of the irradiation products (formed in over 90% yield) of **2** ⟷ **3** at pH ≃ 7 and **5** at pH ≃ 7 and pH 10 strongly supported their identification as **1** and **6**, respectively.[11] This was further confirmed by means of authentic sample HPLC co-injections.

In a recently published report, most of the chemistry, previously discovered in the case of dimer **1**,[9] has been reproduced using short oligonucleotides (8-mers) containing the Tps^4T motif.[12] Interestingly, in this independent study, it was established that oligonucleotides containing thietanes, corresponding to structures **2** and **5**, could be photoreversed to their parent oligonucleotide by irradiation at 254 nm. Clearly, these findings are consistent with the observations described herein, which are based on unambiguous product and yield



Scheme 1 Photoreversion of s^5-(6-4) photoproducts. Stereochemistry at C-5 for **8** was tentatively assigned on the basis of NOE measurements.

characterizations readily accomplished when using medium sized molecules. However, it was indicated that, when incorporated in an oligonucleotide, the s[5]-Dewar photoproduct **4** undergoes photoreversion under the same conditions, with the concomitant formation of an uncharacterized photoproduct in unspecified yields.[12] Actually, evidence has long been available demonstrating that under 254 nm irradiation, the Dewar photoproduct of TpT is transformed into the (6-4) adduct of TpT.[13] For this reason, we were prompted at the outset of our study to examine the photochemical behaviour of the s[5]-Dewar photoproduct **4**.

In our hands, exposure of **4** to 254 nm light resulted in a complex mixture of photoproducts, the composition of which was dependent on the time of irradiation (HPLC and ^{1}H NMR monitoring). At pH 7, whatever the duration of this irradiation, only a very minor amount of Tps[4]T **1** was formed, whereas at pH 10 a small quantity of (6-4) photoproduct **3** was produced (in both cases below 10%). At first glance, the detection of **1** (pH 7) and **3** (pH 10) in the photolysate of **4** might appear to confirm the observations demonstrating the photoreversibility of the s[5]-Dewar photoproduct.[12] However, a prolonged exposure of **4** to 254 nm light (1 h) resulted in its complete transformation to a single new product **8** which was formed in good yield. Spectral data showed **8** to have a pyrimidinone system (λ_{max} 220 and 328 nm and characteristic ^{1}H NMR signals at δ 8.04 and 2.32 corresponding to H-6 and methyl protons of pT). Sulfur elimination was confirmed by observing in its FAB mass spectrum a peak at *m/z* 575 corresponding to $M - H^+ + 2Na^+$ and by the splitting (*J* 7 Hz) of the H-6 (δ 5.27) and the methyl (δ 1.21) proton signals due to coupling with an extra proton (H-5) resonating at δ 3.49. Thus, this series of experiments showed that the s[5]-Dewar photoproduct **4**, upon irradiation at 254 nm, undergoes desulfurization more rapidly than rearomatization to give presumably **7**, which is finally transformed into the corresponding photostable pyrimidinone **8**. This supports the rearrangement, upon irradiation at 254 nm, of Dewar 2-pyrimidinones into their parent 2-pyrimidinone. However, the high propensity of the s[5]-Dewar derivative **4** to desulfurization excludes its use to model the photoreversibility of this type of lesion in DNA as recently proposed.[12]

In summary, these model reactions carried out on dinucleotides closely related to the naturally occurring (6-4) adducts of DNA, demonstrates: (i) that the (6-4) photoproduct **3** can be efficiently photochemically splitted to regenerate **1**, (ii) that this photoreversal process can be accomplished by direct excitation, and (iii) that it implicates the four membered ring intermediate (thietane) that had led to its formation. Finally, the above study represents an experimental attempt showing that the (6-4) photoproduct DNA photolyase might initially convert the (6-4) photoproduct into an oxetane (azetidine) intermediate by way of a *thermal* process as illustrated by the **2** ⟷ **3** equilibrium. Subsequently, the enzyme substrate complex, in accordance with Prakash and Falvey, would undergo *photocycloreversion* to the unmodified bases, either by direct excitation (this work), energy transfer or photosensitized electron transfer.[8] In addition, it was shown that the s[5]-Dewar photoproduct cannot be used to study the photoreversion of this type of DNA photoproduct.

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Footnote

† Monitored by HPLC and ^{1}H NMR spectroscopic analysis. Irradiation experiments (0.35 mM, 10 ml) were carried out in a 2 cm Φ quartz tube in H_2O (pH ≃ 7 and pH 10, at 24 °C) with a TNN 1532 Hanau lamp as the light source. The irradiated solution (10 μl) was injected onto a Nova-Pak C18 3.9 × 150 mm 4 μ column. A 15 min, 1 ml min^{-1} linear gradient of 0–12% acetonitrile in 0.05 M aqueous ammonium acetate followed by a 15 min plateau was used. A photodiode array detector was employed. For preparative HPLC, a Nova-Pak C18 25 × 100 mm 6 μ column was used.

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