Model Studies of the (6−4) Photoproduct DNA Photolyase: Synthesis and Photosensitized Splitting of a Thymine−5,6-Oxetane

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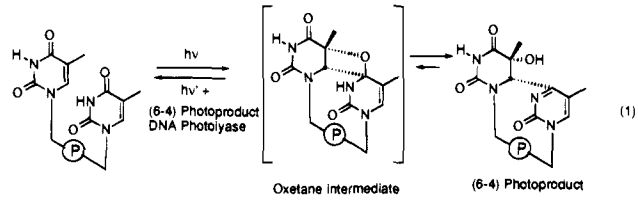
Ultraviolet (UV) light damage to DNA[1−3] and the corresponding enzymatic repair processes[4,5] have been the subject of much recent attention. This is due, in no small part, to concerns about the depletion of stratospheric ozone[6] and the increase in human skin cancer projected to result.[7,8] The most abundant photoproducts from UV irradiation of DNA are the cis-syn pyrimidine dimers (not shown).[3] Most of the experimental studies in this area have focused on the formation and repair of this particular lesion. However, more recent work has suggested that some of the less abundant UV photoproducts might actually be more effective at causing damaging mutations.[8−12] This report is concerned with the (6−4) photoproduct and its repair by a newly discovered enzyme, (6−4) photoproduct DNA photolyase.

The (6−4) photoproduct is an adduct of two pyrimidines that occupy adjacent sites on the same DNA strand.[13−15] In this case the bases are joined by a single C−C bond, and there is a net oxygen transfer between the two rings. Its formation is believed to occur via an initial Paterno−Büchi type cycloaddition to form an oxetane intermediate. The latter rapidly isomerizes through a proton shift coupled with a C−O bond scission (eq 1).[13−15]

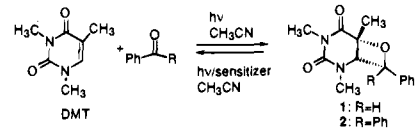


(1)

Recently, Todo et al.[16] reported the discovery of a protein from *Drosophila melanogaster* cell extracts which can effect the photoreversal of (6−4) photoproducts. Like the more familiar pyrimidine cyclobutane dimer DNA photolyases, the new protein binds to the (6−4) lesion in the dark, and upon absorption of a photon, the photoproduct is repaired to the normal base forms. This process formally involves both a C−C bond scission, as well as transfer of an oxygen atom. Kim et al.[17] have proposed an elegant mechanism for the repair process. Their model holds that binding of the lesion to the protein shifts the equilibrium to favor the oxetane. Absorption of a photon (ca. 400 nm) by the enzyme−substrate complex causes cycloreversion through a photosensitized electron transfer mechanism analogous to that reported for the pyrimidine cyclobutane dimer DNA photolyase.[18,19]

We were unaware of any examples of oxetane cycloreversions initiated by single electron transfer. One problem with evaluating the proposed mechanism is that the natural oxetane intermediates are not isolable; they spontaneously undergo the thermal ring opening reaction depicted in eq 1. Therefore we prepared two 5,6 oxetane adducts of 1,3-dimethylthymine (DMT) with benzaldehyde (**1**) and benzophenone (**2**) and examined their behavior under electron transfer conditions. These model compounds are stable in their oxetane form, and their ability to undergo the proposed cycloreversion reaction can be studied without the complication of the ring-opening equilibrium.

Oxetane **1** was prepared by the photolysis of a 15 mL CH_3CN solution containing benzaldehyde (0.16 g) and DMT (0.15 g).[20] The preparation of **2** has been reported previously.[21]



(2)

The oxetanes react with the excited singlet states of the sensitizers listed in Table 1. This was determined by fluorescence quenching experiments. Solutions of the sensitizer were prepared in CH_3CN, purged with N_2, and their fluorescence intensities measured with varying concentrations of added oxetane. The results are illustrated in Figure 1 for *N,N,N′,N′*-tetramethylphenylenediamine (TMPD). In each case the fluorescence intensity decreases as oxetane is added. The $k_q\tau$ values were determined from the slopes of Stern−Volmer plots.[22]

Both electron donors and acceptors are able to photosensitize the splitting reaction. The most efficient reactions are seen with the strongest electron donors, TMPD and *N,N,N′,N′*-tetramethylbenzidine (TMB). These have excited state oxidation potentials (E_{ox}*) of −3.25 and −3.17 V (vs SCE), respectively.[23] Aniline (−3.04 V) and pyrene (−2.14 V) also effect the reaction, although longer irradiation times are required. Pyrene has an unusually long excited state lifetime and also a much broader absorption spectrum than the aromatic amines. This may, in part, compensate for its higher E_{ox}*. A reduced flavin, 9,10-dihydrotetraacetylriboflavin ($(AcO)_4rFH_2$; E_{ox}* = −2.6 V), was also examined. Similar reduced flavins act as the proximate

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(20) The solution was sealed in a quartz tube, purged with N_2, and irradiated with the unfiltered output from a 150 W Hg−Xe lamp for 8 h. Evaporation of the solvent and elution of the residue over silica with 20% EtOAc in hexane gave 0.080 g of **1** as a waxy solid. The *exo* orientation of the phenyl ring was assigned on the basis of a 6.2 Hz NMR coupling constant for the two cyclobutyl protons. ^{1}H NMR: ($CDCl_3$, 200 MHz) δ 1.75 (s, 3H); 2.93 (s, 3H), 3.27 (s, 3H); 3.85 (d, 1H, J = 6.2 Hz); 5.43 (d, 1H, J = 6.2 Hz); (bs, 7.36, 5H). IR: 1712, 1681 cm^{-1}. MS (FAB) : m/z = 261 (M + 1); 155. HPLC analysis showed the compound to be >95% pure.
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Table 1. Splitting Reactions of Oxetanes **1** and **2** with Various Sensitizers[a]

sensitizer	reactant (nmol) oxetane	time (min)	products (μmol) oxetane	PhC(O)R	DMT	$k_q\tau$ (M^{-1})
none[b]	**1** (40)	60	22.2	9.6	13.5	
donors						
TMB	**1** (6.3)	10	1.2	4.0	4.4	220
TMPD	**1** (7.0)	5	0.7	4.9	5.0	41
TMPD	**2** (3.5)	5	1.1	2.3	2.0	38
TMPD[d]	**2** (120)	30	72	21	35	38
$PhNH_2$	**1** (6.7)	60	3.7	2.1	2.9	81
pyrene	**1** (6.7)	120	6.4	0.2	0.3	67
$(AcO)_4rFlH_2$	**1** (6.6)	2100	6.0	0.5	*c*	*c*
acceptors						
DCA	**1** (6.5)	120	4.3	1.0	1.3	17
$(AcO)r_4Fl$	**1** (7.3)	1135	5.8	1.0	0.2	11

[a] Sensitizer and oxetane were prepared as 3 mL, N_2-purged CH_3CN solutions in a sealed quartz cuvette. Samples were irradiated with a 150 W Hg–Xe lamp using a 290 nm cutoff filter. Product distributions were quantified by HPLC. [b] Direct photolysis carried out without a filter. [c] Could not be determined. [d] Determined by ^1H NMR.

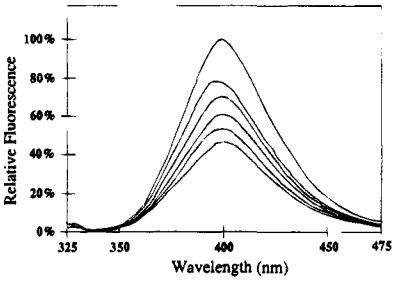

Figure 1. Fluorescence spectra of TMPD in CH_3CN in the presence of 0–5 mM **1**.

sensitizers in some cyclobutane dimer photolyases.[18,19] $(AcO)_4rFH_2$ sensitizes the splitting reaction, but only after prolonged irradiation. Unfortunately, under these conditions the flavin begins to decompose and its photoproducts interfere with analysis of the DMT. However, the aldehyde is detected in reasonable yields.

Electron acceptors also photosensitize the splitting reaction. 9,10-Dicyanoanthracene (DCA; $E_{red}^* = +1.93$ V) and tetraacetylriboflavin ($(AcO)_4Fl$; $E_{red}^* = +2.42$ V) lead to monomeric products. The low yields of the DMT can be attributed to the high reactivity of its cation radical. Direct irradiation (in this case the unfiltered output of the Hg–Xe lamp was employed) also leads to splitting.

We are currently investigating the mechanisms of these splitting reactions in more detail. However, several conclusions can be drawn from the preliminary data. First, singlet and triplet energy transfer mechanisms can be excluded. Fluorescence quenching experiments indicate that the chemistry is initiated from the singlet, rather than the triplet, states of the sensitizers. Singlet energy transfer can be excluded on the basis of energetic grounds. Neither **1** nor **2** shows significant absorbance above 290 nm, and we therefore estimate the singlet energies of oxetanes **1** and **2** at 98 ± 5 kcal/mol. The sensitizers all have singlet energies <80 kcal/mol.[24]

Second, both the reductive and oxidative electron transfer reactions occur initially on the thymine portions of the oxetanes. This is based on the likely redox potentials of the functional groups present in **1** and **2**. The thymine residue has a saturated 5,6 bond and can be considered analogous to the DMT cyclobutane dimer. The latter has a reduction potential of ca. −2.6 V (vs SCE).[23] The unconjugated benzene ring(s) on the aldehyde/ketone portion of the oxetane would be much more difficult to reduce. Benzene has a reduction potential of ca. −3.2 V.[25] By the same reasoning, one-electron oxidation is also expected to occur on the thyminyl portion of the oxetanes. The oxidation potential (E_{ox}) of the DMT cyclobutane dimer is ca. +1.5 V[26] whereas that of benzene is +2.9 V.[25] The other possible site for electron abstraction would be the oxetane oxygen. An oxidation potential of +2.5 V is commonly cited for diethyl ether.[27] Therefore the pyrimidine ring represents the most energetically favorable location for single-electron reduction or oxidation.

The photochemical behavior of the thymine–5,6-oxetanes is in many ways reminiscent of the previously characterized thymine–thymine,[28–31] thymine–cytosine,[32] and cytosine–cytosine cyclobutane dimers.[32] Cycloreversion (i.e., splitting) of these oxetanes can be promoted either by direct irradiation or by sensitization with either electron donors or acceptors.

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