Oxidative Splitting of a Pyrimidine Cyclobutane Dimer: A Pulse Radiolysis Study

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The oxidative splitting process of *cis-syn* 1,3-dimethyluracil cyclobutane dimer (DMUD) in aqueous solution was investigated using pulse radiolysis technique. The results indicated that DMUD can be splitted into 1,3-dimethyluracil (DMU) by OH radicals (OH$^{\cdot}$) and Br$_2$ radical anions (Br$_2^{\cdot-}$), but not by azide radicals (N$_3^{\cdot}$). The oxidative mechanisms that an H-abstracted from DMUD for OH$^{\cdot}$ oxidative splitting and an electron transfer from DMUD to Br$_2^{\cdot-}$, were suggested. Related kinetic parameters were determined.

Keywords   pyrimidine cyclobutane dimer, oxidative splitting, pulse radiolysis

Introduction

The pyrimidine cyclobutane dimer (pyr < > pyr) is the most important product formed between two adjacent pyrimidine bases in DNA through a [2 + 2] photocycloaddition upon exposure of UV radiation. This damage can lead, if not repaired, to mutations or cancerogenous cell growth.[1-3] The repair of pyr < > pyr in bacteria is achieved by the dimer specific repair enzyme photolysis through a direct one-electron reduction followed by reversion of the resultant unstable cyclobutane radical anion and subsequent oxidation to the pyrimidine monomers.[4,5] It was shown that cleavage of the cyclobutane pyrimidine dimer could also proceed through an oxidative pathway, since the cyclobutane radical cation was similarly unstable.[6] Molecular orbital calculations predict that both the pyr < > pyr radical cation and anion are prone to decay to their constituent monomers by ring splitting. This oxidative repair was recently used by Barton's group as a mechanistic probe to examine the long-range charge transfer through the DNA base stack.[7-9]

For the model systems of chloranil, anthraquinone sulfonate and dimethylaniline, all of which are powerful oxidants in their excited states, the efficient dimer splitting has been observed.[10,11]

The oxidative cleavages of *cis-syn* cyclobutane thymine dimer (TT) and stereoisomeric C5—C5′ linked thymine dimers by other electrophilic radicals, e. g., the hydroxyl radical (OH$^{\cdot}$), the sulfate radical (SO$_4^{\cdot-}$) and azide radical (N$_3^{\cdot}$), respectively, have been described in recent literature, and both a one-electron oxidation and a hydrogen atom abstraction have been suggested as the primary reaction step.[12-14]

Kruger et al.[15] studied the oxidative splitting of four stereoisomeric uracil cyclobutane dimers by nitrate (NO$_3^{\cdot}$) in an acetonitrile solution, and pointed out that the rate of the splitting reaction and the splitting efficiency, as well as the distribution of the byproducts depended on the stereochemistry and the substitution pattern at the cyclobutane ring significantly. However, the reason of this behavior has not been known yet. Therefor extensive studies on the mechanism of oxidative splitting of pyrimidine dimers are needed.

In this work the dimer splitting by oxidants was studied. To eliminate complications due to coproducts in the photochemical system, a starting point in our study is on the OH$^{\cdot}$ and Br$_2^{\cdot-}$ generated by pulse electron-beam radiolysis reaction with pyrimidine cyclobutane dimers, using *cis-syn* 1,3-dimethyluracil cyclobutane dimer (DMUD) as the simple model compounds.

Experimental

Materials

Cis-syn 1,3-dimethyluracil cyclobutane dimer (DMUD) was prepared by the photo-dimerization of 1,3-dimethyluracil (DMU) using acetone as photosensitizer, followed by separation of isomers by silica gel column chromatography.[16] ^{1}H NMR (400 Mz, DMSO-d_6) δ: 2.84 (s, 6H), 2.91 (s, 6H), 3.77 (dd, $J = 2.4$ Hz, $J = 9.6$ Hz, 2H,), 4.11 (dd, $J = 2.4$ Hz, $J = 9.6$ Hz, 2H) were obtained. Potassium bromide (KBr) and phosphates (analytic grade) were ob-

tained commercially and used as received.

Unless otherwise indicated, all solutions were freshly made with triply distilled water buffered with 0.1 mol $\cdot$ L^{-1} phosphate at pH 7 and were saturated with high purity nitrous oxide ($>99.99\%$) before taking part in pulse radiolysis experiments to enable the conversion of hydrated electron into OH radical. All experiments were performed at room temperature.

Pulse radiolysis experiments

Pulse radiolysis experiments were performed utilizing a 10 MeV liner accelerator, which delivers an electron pulse with duration of 8 ns. The analyzing light beam passed perpendicularly through a 2-cm quartz cell. The transmitted light entered a monochromator equipped with a R955 photomultiplier. The signals were collected using an HP54510B 300 MHz transient recorder and then processed with a personal computer. The dosimetry of electron pulse was determined by a thiocyanate dosimeter using G [$(CNS)_2^{\cdot-}$] $= 2.9$ in a 10 mmol $\cdot$ L^{-1} KCNS solution in air by taking $\varepsilon_{480} = 7600$ $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ for $(CNS)_2^{\cdot-}$. Detailed descriptions of the equipment and experimental conditions were given elsewhere.[17] In this work, the dose per electron beam pulse was about 10 Gy.

Results and discussion

The radiolysis of dilute aqueous solution saturated with N_2 leads to the production of three primary highly reactive intermediates e_{aq}^-, OH and H besides other less reactive molecular compounds H^+, H_2 and H_2O_2. Under the present conditions of radiolysis, more than 98% of hydrated electrons are converted into OH radicals by N_2O [Eq. (2): $k(e_{aq}^- + N_2O) = 9.1 \times 10^9$ $dm^3 \cdot mol^{-1} \cdot s^{-1}$].

$$H_2O \to e_{aq}^- + OH + H \ etc. \qquad (1)$$
$$e_{aq}^- + N_2O \to N_2 + OH + OH^- \qquad (2)$$
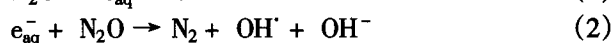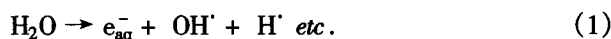
Reaction of OH with DMUD

The OH radical has a high reduction potential [E_{red} (OH/OH$^-$) $= 2.7$ V vs. NHE],[18] however, its reaction with organic substrates in aqueous solution rarely, if ever, proceeds by direct electron transfer, but generally occurs by either the addition at unsaturated bonds or the abstraction of H-atoms. Cleavage of DMUD by OH, produced via Eqs. (1) and (2), was carried out in N_2O-saturated 0.2 mmol $\cdot$ L^{-1} DMUD aqueous solution. The transient absorption spectrum recorded at 0.5, 5 and 50 μs after pulse radiolysis is displayed in Fig. 1, and two absorptions at 350 and 390 nm are observed. Inset of Fig. 1 shows the growth trace of the transient absorbances at 350 and 390 nm. The bimolecular rate constant 2.21×10^9 $dm^3 \cdot mol^{-3} \cdot s^{-1}$ for the reaction of

OH and DMUD was derived from the first-order build-up of the transient absorbance observed at 390 nm by changing the concentration of DMUD in the range of 0.01—0.08 mmol $\cdot$ L^{-1}. The reaction of the OH and DMUD may proceed as OH abstracting an H-atom from DMUD and giving DMUD$^{\cdot+}$. The last product can be explained by DMUD$^{\cdot+}$ followed by hydrolytic splitting.
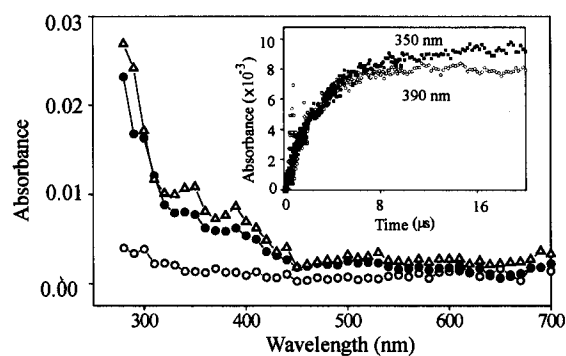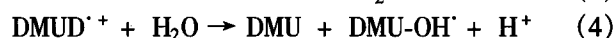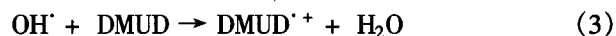
$$OH + DMUD \to DMUD^{\cdot+} + H_2O \qquad (3)$$
$$DMUD^{\cdot+} + H_2O \to DMU + DMU\text{-}OH + H^+ \qquad (4)$$

Fig. 1 Transient absorption spectra obtained in the pulse radiolysis of N_2O-saturated aqueous solution containing 0.2 mmol $\cdot L^{-1}$ DMUD recorded at (○) 0.5 μs, (●) 5 μs and (△) 50 μs after the electron beam pulse. Insert: the time dependence on the absorbances at 350 and 390 nm.

By analogy with 5,6-dihydrothymine[19] H-abstraction from C-6 (C-6′) might be expected to be of major importance (Scheme 1). The absorptions at 350 and 390 nm maybe come from two stereoismeric products, respectively.

Reaction of $Br_2^{\cdot-}$ and DMUD

Oxidizing Br_2 radical anions [$Br_2^{\cdot-}$; $E_{red}(Br_2^{\cdot-}/2Br^-)$ $= 1.7$ V vs. NHE][18] were generated by γ-radiolysis of N_2O-saturated phosphate buffer solution containing an excess amount of KBr (50 mmol $\cdot$ L^{-1}) to examine the oxidative splitting reactivity of pyrimidine cyclobutane dimers. The OH radicals are in turn scavenged by Br^- ions to produce oxidizing Br_2 radicals.

$$Br^- + OH \to Br + OH^- \qquad (5)$$
$$Br + Br^- \to Br_2^{\cdot-} \qquad (6)$$

The transient absorption spectra recorded at 1.0, 10 and 20 μs after pulse radiolysis of N_2O-saturated 0.2 mmol $\cdot$ L^{-1} DMUD aqueous solution containing 50 mmol $\cdot$ L^{-1} KBr are shown in Fig. 2. First reaction is that hydrated electron converted into OH radical, then OH radical reacts with Br^- and $Br_2^{\cdot-}$ is given. So the transient absorption spectra recorded at 1.0 μs after the electron pulse stem is from $Br_2^{\cdot-}$ predominately. The absorption at 360 nm is characteristic of $Br_2^{\cdot-}$.[20]

Scheme 1
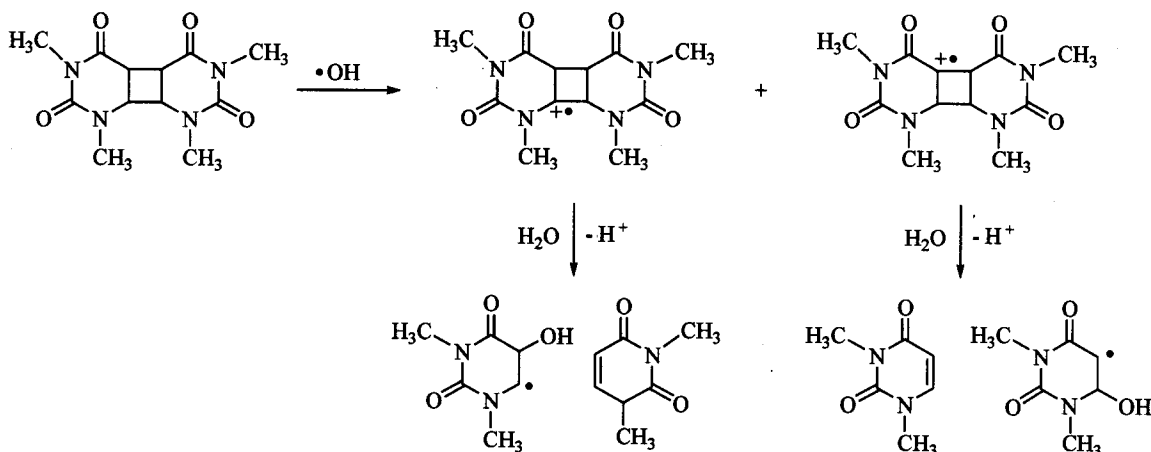
Following the rapid decay of $Br_2^{\cdot-}$ observed at 360 nm, a concomitant increase of absorbance at 270 nm has been observed. Inset of Fig. 2 shows the trace curves of the decay at 360 nm and the growth at 270 nm. Based on synchronization between two curves, it is obvious that DMUD was oxidized into $DMUD^{\cdot+}$, and the very unstable $DMUD^{\cdot+}$ splitted into DMU and $DMU^{\cdot+}$ rapidly within 0.2 μs of the pulse to escape direct detection. So the growth at 270 nm has been assigned to the absorbance of $DMU^{\cdot+}$. By varying DMUD concentrations in the range of 0.1—0.6 $mmol \cdot L^{-1}$, the rate constant $9.69 \times 10^7 \ dm^3 \cdot mol^{-1} \cdot s^{-1}$ for the one electron transfer from DMUD to $Br_2^{\cdot-}$ has been determined from the pseudo-first-order decay kinetics observed at 360 nm.

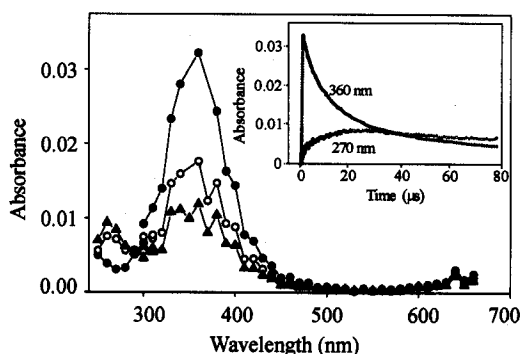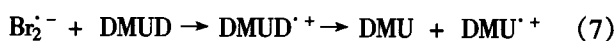$$Br_2^{\cdot-} + DMUD \rightarrow DMUD^{\cdot+} \rightarrow DMU + DMU^{\cdot+} \qquad (7)$$

Fig. 2 Transient absorption spectra from pulse radiolysis of N_2O-saturated aqueous solution containing 0.2 $mmol \cdot L^{-1}$ DMUD and 50 $mmol \cdot L^{-1}$ KBr (pH 7) at (●) 1.0 μs, (○) 10 μs, (▲) 20 μs after pulse. Insert: growth trace of the transient absorbance observed at 270 nm and decay curve recorded at 360 nm.

Voityuk *et al.*[21] led a quantum chemical study of photoinduced DNA repair using the AM1 UHF method, and the results for the dimer cation radical favor a splitting reaction by the cleavage of the C6—C6' bond first.

Rak *et al.*[22] has studied the cation pathway of the cyclobutane-type uracil dimer cycloreversion using various quantum chemical methods. Hartree-Fock (HF) calculations

showed that the reaction pathway for the splitting process was associated with the conformational flexibility of the intermediate in which the two uracil rings were connected by the remaining C5—C5' bond.

So the detailed mechanism of $Br_2^{\cdot-}$ reacting with DMUD can be deduced as Scheme 2.

Scheme 2
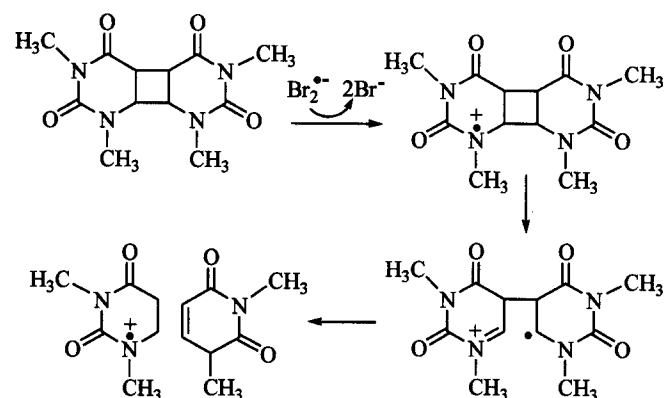
Using azide ions (N_3^-; 50 $mmol \cdot L^{-1}$ NaN_3) in stead of Br^- ions in N_2O-saturated phosphate buffer, oxidizing azide radicals [$N_3^{\cdot}$; $E_{red}(N_3^{\cdot}/N_3^-) = 1.3$ V vs. NHE][18] were also generated as shown in Eq. (8) ($k = 1.2 \times 10^{10} \ dm^3 \cdot mol^{-1} \cdot s^{-1}$).

$$N_3^- + OH^{\cdot} \rightarrow N_3^{\cdot} + OH^- \qquad (8)$$

In contrast to the reactivity toward OH radicals and Br_2 radical anions, DMUD can not be split into DMU by less oxidizing azide radicals. Oxidation potential of $N_3^{\cdot}$ is too low to oxidate DMUD.

We have demonstrated that oxidative splitting of DMUD into DMU by oxidizing radicals $OH^{\cdot}$ and $Br_2^{\cdot-}$ generated in the radiolysis. The results of DMUD show great difference to those of TT,[13] and TT can be oxidized by $OH^{\cdot}$ and $SO_4^{\cdot-}$, but not by $Br_2^{\cdot-}$. The oxidation potential of TT is close to that of DMUD, so the difference may due to the different stereochem-

istry and the substitution pattern at the cyclobutane ring of DMUD and TT.

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