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Steady-state and laser flash photolysis studies on the oxidative splitting of cyclobutane thymine dimer by triplet 9,10-anthraquinone-2-sulfonate

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Abstract

The oxidative splitting reaction of cyclobutane dimethylthymine dimer (DMTD) by triplet excited state of 9,10-anthraquinone-2-sulfonate (^{3}AQS*) was investigated using 355 nm laser flash photolysis technique combined with steady-state analysis. The transient absorption spectra for interaction of ^{3}AQS* with DMTD in aqueous solution were observed. Comparing to the transient absorption spectra of pure anthraquinone-2-sulfonate aqueous solution reported previously, two new absorption bands from photolysis of AQS aqueous solution containing DMTD were assigned to the absorption of AQS radical anion and dimethylthymine monomer (DMT) radical cation, respectively. A splitting reaction of DMTD via electron transfer to ^{3}AQS* was demonstrated, and the quenching rate constant of ^{3}AQS* by DMTD was determined. Furthermore, steady-state photolysis results show that ^{3}AQS* initiates the oxidative splitting reaction.

Keywords: Laser flash photolysis; 9,10-Anthraquinone-2-sulfonate; Oxidative splitting; Dimethylthymine dimer; Radical cation

1. Introduction

It is well known that DNA damage is induced by chemical oxidants, ionizing radiation and UV light [1,2]. The cyclobutane pyrimidine dimers, which result from [2 + 2] photo-cycloaddition between 5,6 C=C double bonds of two adjacent pyrimidines in the same strand of DNA, are the most abundant photo-products among the major three types of photo-induced DNA damage [3,4]. Although the reverse photo-chemical reaction is symmetry allowed by orbital symmetry consideration, the dimers do not significantly absorb UVB (290–320 nm) light as they do not possess the conjugated π-systems of the original pyrimidines, and thus dimers accumulate in DNA.

Repair of the DNA damage occurs either by photo-reactivation through concurrent or subsequent exposure to near-UV and visible light, or by nucleotide- or base-excision repair pathways [5–8]. In the photo-reactivation process, a commonly accepted model for the repair mechanism proposes that the dimer splitting is a consequence of a single electron transfer from the excited cofactor $FADH^-$ to the dimer. The dimers could be repaired through photo-induced electron transfer to or from the photo-sensitizers [9,10].

In model study of photo-reactivation mechanism, it is found that photo-sensitized splitting reaction can occur at the wavelength longer than the direct photo-fragment required [11]. As demonstrated by photo-chemical and radiation-chemical studies, the splitting reaction of photo-dimers could also occur by a one-electron oxidation mechanism involving appropriate oxidants such as sulfate radical anion ($SO_4^{\bullet -}$), OH radical ($^\bullet$OH), and photo-excited 9,10-anthraquinone-2-sulfonate (AQS*) [12–14]. The oxidative splitting mechanism was thought to be distinct from the one-electron reduction mechanism [15–18], where the photo-dimer radical cation underwent facile splitting at the C6–C6′ bond in the initial step and, successively, at the C5–C5′ bond. Such a mechanistic difference in the splitting of photo-dimers was supported by recent computational studies based on the semiempirical AM1 and ab initio HF and MP2 methods [19,20].

Direct spectroscopic evidence for the existence of photo-sensitizer radical ions had importance for understanding

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the mechanisms of dimer splitting by photo-sensitizers [21–23], including enzymatic systems. In systems consisting of dimers and electron donors, detection of the donor radical cations as a transient intermediate likewise implied the existence of the dimer radical anion. The tendency of dimer radical anions to split had been rationalized on the basis of simple molecular orbital theory [24,25].

The dimer radical cation may be an intermediate in dimer splitting that was photo-sensitized by electron abstractors, such as metal ions and quinines [26,27]. Using photo-CIDNP technique, Young et al. [13] found evidence that dimer radical cations exist and are intermediates in anthraquinone sulfonate-sensitized pyrimidine dimer splitting. However, the detailed reaction mechanism from the spectroscopic research was deficient.

This paper reported photo-sensitized one-electron oxidation of the cyclobutane dimethylthymine dimer (DMTD) by ^{3}AQS*, using 355 nm laser flash photolysis technique. The anthraquinone-2-sulfonate (AQS) radical anion and 1,3-dimethylthymine monomer radical cation were observed in our transient absorption spectra. Along with transient photolysis study, the product study was also performed to investigate the splitting effect of DMTD.

2. Experimental details

2.1. Sample preparation

cis-syn-1,3-DMTD was prepared as reported previously [28]. AQS was purchased from Fluka and used as received. $MnCl_2$ (analytic grade reagent) was recrystallized from triply distilled water. The resulting mixtures were bubbled with high-purity argon (99.999%) for 20 min before the spectroscopic measurements were performed. Triply distilled water was used as the solvent and all the spectra were recorded in ambient temperature at pH 5.0.

2.2. Laser flash photolysis

The instrumentation and experimental procedures used for laser flash photolysis were reported previously [29]. The excitation light was the third harmonic (355 nm) of a Nd:YAG laser (Spectra Physics, GCR-170, repetition rate: 10 Hz) with a duration of 8 ns. The analyzing light was from a 500 W Xenon lamp. The laser and analyzing light beams, crossed at right angles, passed through a quartz cell with an optical path length of 10 mm. A monochromator (MC-30N, Ritsu Oyo Kogaku) equipped with a GDB 59 photo-multiplier was used to analyze transient absorption. The signals from the photo-multiplier were displayed and recorded as a function of time on a Tektronix TDS 380 oscilloscope. Each data point was obtained with multi-times average to improve the signal-to-noise ratio. The transient absorption spectra were obtained from a series of oscilloscope traces measured with the same solution in a point-by-point manner with respect to the wavelength.

2.3. Photo-product analysis

The DMTD aqueous solution at different concentration were prepared in a quartz cuvette, to which equal molar concentration of the sensitizer was added to give an absorbance value close to 0.5 at 355 nm. The cuvette was then sealed and purged with Ar for 20 min, and irradiated with the output of a 355 nm Nd:YAG laser with a laser power of 45 mW at a frequency of 10 Hz. Five milliliters photolyzed solutions were analyzed by UV to determine the yield of the products after photolysis.

UV spectra were measured on a UV-Vis (Shimadzu UV-2100) spectrophotometer.

3. Results and discussion

3.1. Transient absorption spectra and kinetics from photolysis of AQS aqueous solution in the presence of DMTD

The transient absorption spectra from photolysis of 0.41 mM AQS aqueous solution, at different delay times after laser pulse, were obtained as shown in Fig. 1. There were three different absorption bands at 500 ns after laser pulse, and their peaks were 390, 490 and 580 nm, respectively. At 1 μs, the absorption peaks at 390 and 580 nm decreased obviously, and disappeared completely at 10 μs. It is known that three characteristic absorption peaks of the excited triplet state of AQS are 380, 460 and 580 nm, and its lifetime is shorter than 1 μs [30,31]. The peak shift from 460 nm to 490 nm in our results, at 500 ns delay time, was

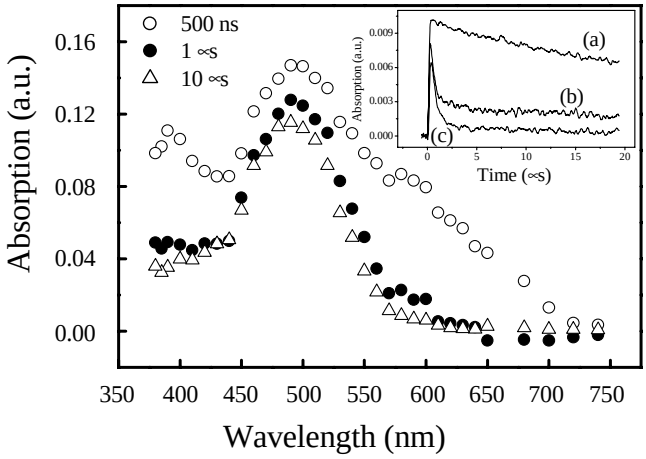


Fig. 1. Transient absorption spectra from the laser photolysis of 0.42 mM anthraquinone-2-sulfonate aqueous solution saturated with argon at 500 ns (○), 1 μs (●) and 10 μs (△). Inset: transient traces observed upon the photolysis of 0.42 mM AQS aqueous solution saturated with argon: recorded at (a) 490, (b) 390, and (c) 580 nm.

due to the long-lived species (symbolized as "B") resulting from AQS–water interaction, which had a broad absorption peak at around 480–490 nm [32]. Our result at 10 μs was the pure absorption spectra of the long-lived species "B" in agreement with Loeff et al. [32].

From the time profiles at different peaks in the inset of Fig. 1, we could see that only the absorption band at 580 nm was not influenced by species "B", so we assigned transient absorption at 580 nm only resulting from ^{3}AQS*. Decay trace at 390 nm was not followed by first-order kinetic law as it comprised two processes: the fast decay resulting from ^{3}AQS* and the slow decay combining long-lived species "B". The transient absorption spectrum of hydrated electron with a characteristic peak around 700 nm was not observed. It ascertained that AQS could not be ionized by 355 nm laser light.

In the presence of DMTD, which has no absorption at 355 nm, the transient absorption spectra of photolysis of AQS solution were observed (Fig. 2). In contrast to Fig. 1, at 500 ns, the three absorption peaks of ^{3}AQS* were replaced by two new absorption peaks around 410 nm and 510 nm. Loeff et al. [33] suggested that the species "B" did not participate in the electron-transfer reaction and just decayed through reverting back to starting material. So the transient absorption spectra of species "B", with or without DMTD, were almost invariant at fixed delay time. Thus, the difference spectra could be obtained by subtracting the spectra of pure AQS from photolysis of AQS solution with DMTD as shown in Fig. 3. Two distinct peaks appeared around 410 and 510 nm in the difference spectra. These two new peaks should be originated from new species rather than ^{3}AQS* and species "B".

The transient absorption spectrum characterized by λ_{max} at 510 nm (Fig. 3) was in good agreement with that of AQS radical anion reported previously [33,34].

Takeo et al. [34] pointed out that AQS radical anion had two distinct peaks around 500 and 385 nm in aqueous solution. From our experiment, the new peak around 410 nm could not be originated from ^{3}AQS*, long-lived species "B"

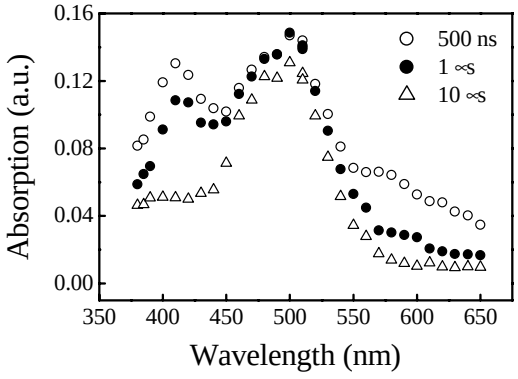


Fig. 2. Transient absorption spectra from the laser photolysis of 0.42 mM anthraquinone-2-sulfonate aqueous solution containing 0.5 mM DMTD saturated with argon at 500 ns (○), 1 μs (●), and 10 μs (△).

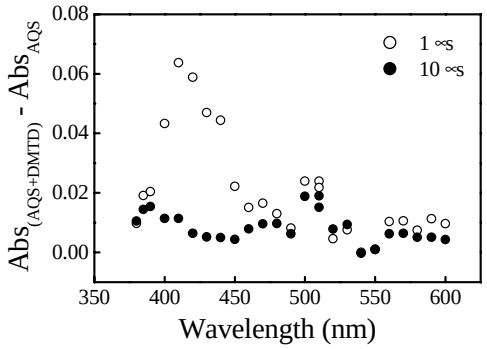


Fig. 3. The difference spectra between the transient absorption spectra of AQS (0.42 mM) with DMTD (0.5 mM) aqueous solution and pure AQS (0.42 mM) aqueous solution saturated with argon at 1 (○) and 10 μs (●).

and AQS radical anion, but belong to the reaction intermediate of DMTD oxidized by ^{3}AQS*.

As we knew, after losing an electron, dimer radical cation would split within 200 ns of the laser pulse [13], and produce one thymine monomer and one thymine monomer radical cation rapidly. This process is too fast to be observed in our experiment, so the absorption at 410 nm is not attributed to DMTD radical cation.

Using the pulse radiolysis technique with optical and conductance detection, Deeble et al. [35] investigated the reaction of $SO_4^{\bullet -}$ with thymine derivatives. They found that 1,3-dimethylthymine radical cation with absorption peak around 400 nm has a lifetime of about 2 μs even at pH 10.1. From Fig. 3, it is obvious that the peak of 410 nm, recorded at 1 μs, fully disappeared at 10 μs instead of a new peak around 390 nm, while the peak of 510 nm was invariable. It implied that the lifetime of the intermediate at 410 nm was shorter than 10 μs. The result is consistent with the observation from Deeble's group [35]. So the 410 nm peak could be ascribed to dimethylthymine radical cation. At the same time, the difference spectrum at 10 μs was in agreement with the spectrum of pure AQS radical anion holding two peaks around 500 and 385 nm [34], thereby confirming the existence of AQS radical anion.

It has been demonstrated that there are two decay pathways for intermediate pyrimidine radical cation: nucleophilic addition and one-electron reduction. In general, the decay channels of the intermediate pyrimidine radical cations are strongly controlled by the substituent at the N(1) and N(3) positions [36]. If the substituent is a hydrogen atom at the N(1) or N(3) position, the deprotonating reaction dominates. Otherwise, the nucleophilic addition of OH^- at the C(5) and/or C(6) is dominating by creating neutral radical. For our DMT radical cation, all substituents at the N(1) and N(3) are methyl groups, so the deprotonating effect at the N(1) and N(3) was negligible. Since the pH of our aqueous solution was around 5.0, it meant that the concentration of OH^- was just about 10^{-9} dm^3 mol^{-1}. In such low OH^- concentration, the nucleophilic addition reaction could also be neglected. Hence, a back electron-transfer reaction from

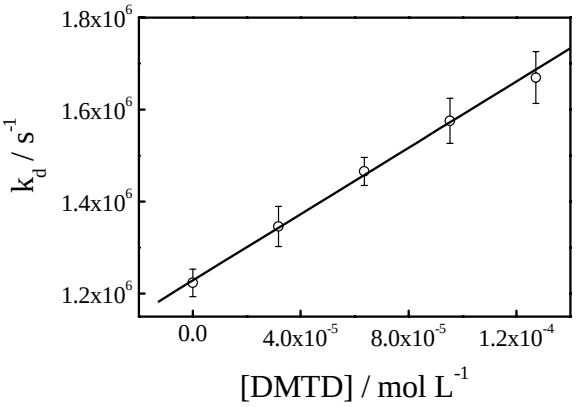


Fig. 4. Plot of the decay rate constants of excited triplet state AQS vs. the concentration of DMTD aqueous solution from the 355 nm laser photolysis ([AQS] = 0.44 mM).

AQS radical anion to pyrimidine radical cation should be the most possible decay pathway of pyrimidine radical cation, and should produce a pyrimidine [34].

The variation of decay rate constant of ^{3}AQS* with the increment of DMTD were obtained as shown in Fig. 4, and the decay kinetics of ^{3}AQS* fit well the first-order kinetics. The quenching reaction by DMTD was so drastic that we could determine the quenching rate constant of ^{3}AQS* by DMTD in that low concentration region at 580 nm: $k_{q(AQS)} = (3.53 \pm 0.10) \times 10^9\ M^{-1}\ s^{-1}$. Obviously, the rate constant for the quenching reaction by DMTD was close to the modified [37] diffusion-limited encounter frequency, which was also the electron-transfer rate constant of such reaction.

Moreover, the free energy change (ΔG, kcal/mol), for excited triplet state AQS reacting with DMTD, was calculated according to the Rehm–Weller equation [38].

$$\Delta G\ (\text{kcal mol}^{-1}) = 23.06 \times \left(E_{ox} - E_{red} - \frac{e^2}{\varepsilon d} \right) - \Delta E_{0,0} \quad (1)$$

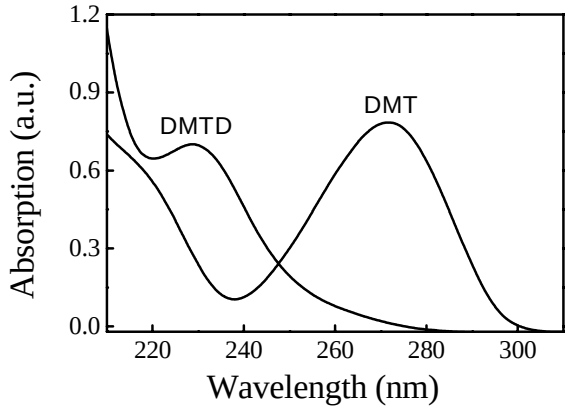


Fig. 5. UV-visible absorption spectra of 1,3-dimethylthymine dimer, 1,3-dimethylthymine monomer in aqueous solutions ([DMTD] = [DMT] = 0.1 mM).

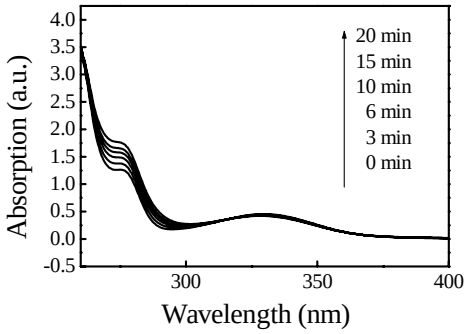


Fig. 6. UV spectra of AQS with DMTD upon the irradiation of 355 nm laser at different time.

The $e^2/\varepsilon d$ could be neglected in aqueous solution. AQS possesses a reduction potential of −0.39 V versus NHE [39]; the oxidation potential for the thymine dimer in DNA is about 1.70 V versus NHE [40]. The electronic excitation energy of the triplet AQS is 2.68 eV [41], which is equal to 61.80 kcal/mol. The free energy change (ΔG) is −13.6 kcal/mol for this electron-transfer reaction. We could see that the electron-transfer reaction of AQS with DMTD is thermodynamically feasible.

3.2. Steady-state experiments

Steady UV absorption spectra of DMTD and DMT were obviously different as shown in Fig. 5. The UV absorption maximum of 1,3-dimethylthymine is at 272 nm, which is far away from the UV absorption maximum of the dimer in

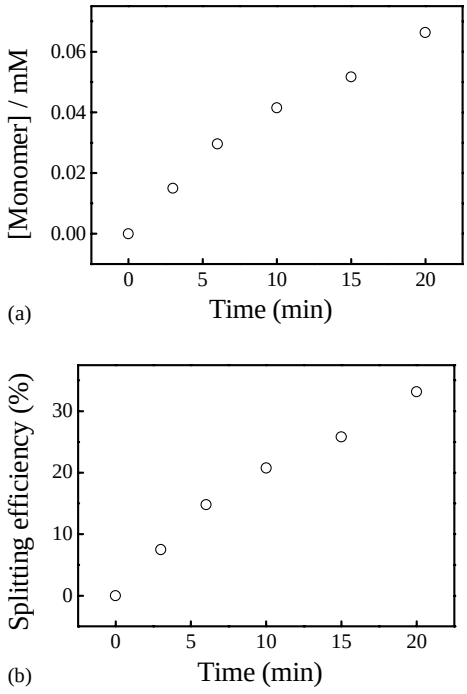


Fig. 7. (a) The DMT monomer yields under different irradiation time. (b) The splitting efficiencies of DMTD under different irradiation time ([AQS] = 0.083 mM and [DMTD] = 0.10 mM).

aqueous solution. Furthermore, the difference between the molar extinction coefficient of the monomer and dimer offered great benefit on using UV spectra for product analysis ($\varepsilon_{272(DMT)} = 7.85 \times 10^3\,M^{-1}\,cm^{-1}$, $\varepsilon_{272(DMTD)} = 0.14 \times 10^3\,M^{-1}\,cm^{-1}$). The extent of splitting was measured by following the increase in UV absorption due to the formation of pyrimidine monomer, over a period of time of irradiation.

Fig. 6 showed the UV spectra of AQS with DMTD upon the irradiation of 355 nm laser at different times. It was apparent that the absorption peak at about 330 nm, which was the maximal characteristic absorption band of AQS ground electronic state, basically had no change, so no chemical reaction occurs for AQS. While near 272 nm, the trend of variation was the most evident. For the reaction of AQS with DMTD irradiated by 355 nm laser, partial DMTD was turned into DMT evidently, while AQS was unchanged. All of these confirmed that AQS just was a "catalyst" in the reaction of DMTD splitting.

The yields of DMT and splitting efficiencies of DMTD under different irradiation time were determined from the variation of the absorption at 272 nm as shown in Fig. 7. It was evident that splitting efficiency of DMTD ($([DMT]/2[DMTD]_0) \times 100\%$) and yield of DMT increased constantly over time, herein, [DMT] represents the concentration of DMT produced after the irradiation of 355 nm laser, while $[DMTD]_0$ represents the concentration of DMTD before the irradiation.

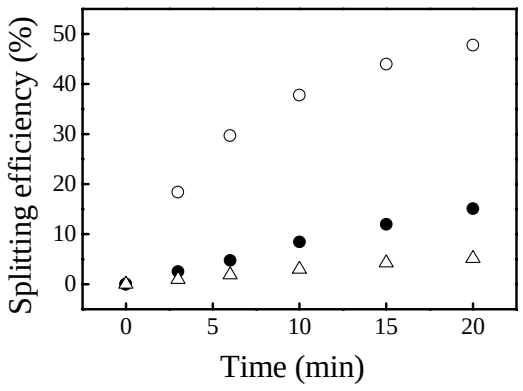
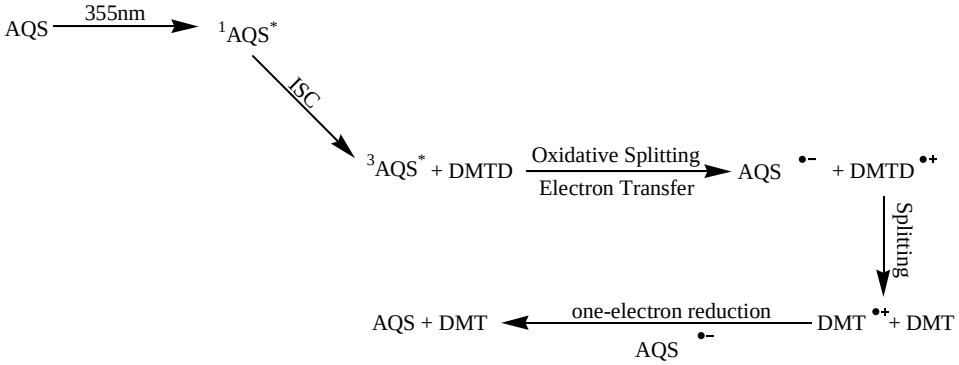


Fig. 8. Changes of the splitting efficiencies of AQS (0.083 mM) with DMTD (0.3 mM) at different irradiation time by 355 nm laser adding different concentration of Mn^{2+} (0 (○), 50 (●), and 200 mM (△)).

In Fig. 8, we added excess amount of Mn^{2+}, which does not have any absorbance at 355 nm, into the aqueous solution of AQS with DMTD. Abundant Mn^{2+} accelerate the quenching process of the excited triplet state AQS, thereby in competition with the oxidative splitting reaction. We found the splitting efficiency differed significantly with or without Mn^{2+}. Therefore, the results also confirmed that $^3AQS^*$ initiates the splitting reaction of DMTD.

As a result, the detailed mechanism of the oxidative splitting reaction can be summarized as follows:



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