Photoinduced reaction of excited triplet 9,10-anthraquinone quenched by antioxidant vitamin C

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Abstract. The quenching of the photoinduced excited triplet of 9,10-anthraquinone ($^3AQ^*$) by Vitamin C (VC) has been studied in ethylene glycol-water (EG-H$_2$O, EG/H$_2$O=9/1, v/v) homogeneous by using time-resolved electronic paramagnetic resonance (TR-EPR) and laser flash photolysis techniques. According to the chemically induced dynamic electron polarization (CIDEP) and transient absorptive spectra, the reaction intermediates and the reaction mechanism have been analyzed. The rate constant for the quenching of excited triplet $^3AQ^*$ by VC has been measured. The results indicate that $^3AQ^*$ can capture hydrogen atom not only from ethylene glycol molecule but also from VC. The strong CIDEP signal of VC monoanion radical indicates that the quenching of VC to $^3AQ^*$ is obvious. The rate for the quenching of $^3AQ^*$ by VC is nearly diffusion-controlled.

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Key words: 9,10-anthraquinone, Vitamin C, time-resolved electron paramagnetic resonance (TR-EPR), transient absorptive spectrum, chemically induced dynamic electron polarization (CIDEP)

1 Introduction

Biologic quinones such as 1,4-benzoquinone (PBQ), duroquinone (DQ), 1,4-naphthoquinone (NQ) are widely distributed in biological systems and play important physiological roles as redox carrier in processes such as photosynthesis, oxidative phosphorylation and mitochondrial electron transport [1,2]. However, these biologic quinones are photosensitive. The excited triplet quinones produced by UV light irradiation may produce serious damage to biological tissues in the skins of human. On the one hand, the excited triplet quinones may abstract hydrogen atom from protein, nucleic acid and lipid components

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of cells directly [3]. On the other hand, the excited triplet quinones may oxidate nucleic acid and lipid components of cells by sensitizing ground state oxygen $^3O_2$ to yied highly oxidative singlet oxygen $^1O_2$ indirectly [4,5]. Just for these reasons, the photoinduced reaction of the excited triplet biological quinones quenched by antioxidant have been paid much interest in recent years.

Figure 1: Molecular structures of VC, AsH^- and AQ.

Vitamin C (VC, ascorbic acid, AsH_2, Fig. 1) is one kind of naturally available biological antioxidants contained in vegetables and fruits. There have many reports that VC functions synergistically with Vitamin E (α, β, γ and δ-Tocopherols, TocH) in the biological antioxidation protection process by reducing tocopheroxyl radical $Toc^\bullet$ and to reproduce TocH in vivo [6-9]. However, the direct reactions of the excited triplet $^3DQ^*$ and ectogenic 9,10-anthraquinone ($^3AQ^*$) and quenched by VC have also been reported [10,11].

9,10-anthraquinone (AQ, Fig. 1) is one classical quinone molecule with three benzene ring. Many important drugs which collectively called anthracenediones include 9,10-anthraquinone derivatives. Photoinduced excited triplet 9,10-anthraquinone ($^3AQ^*$) may capture electron from electron donor such as triethylamine to produce 9,10-anthraquinone anion radical $AQ^{\bullet-}$ and triethylamine cation [12]. Excited triplet $^3AQ^*$ also can abstract hydrogen atom from hydrogen donor such as ethylene glycol (EG) to produce neutral 9,10-anthraquinone radical $AQH^\bullet$ and ethylene glycol ketyl radical [13]. In addition, stable radical 2,2,6,6-tetramethylpiperidoxyl (TEMPO) is also an effective quencher of photoinduced $^3AQ^*$ [14]. However, the photoinduced reaction of antioxidant VC to quench $^3AQ^*$ have not been reported up to now.

Time-resolved electron paramagnetic resonance (TR-EPR) and transient absorptive spectrum techniques are both powerful tools for detecting and identifying short-lived intermediate radicals directly in photophysical and photochemical reactions. The chemically induced dynamic electron polarization (CIDEP) signal observed by TR-EPR and transient absorptive spectrum observed by laser flash photolysis can provide significant information about photochemical reaction mechanisms and reaction dynamics. In the present work, we will report the photoinduced reaction mechanism and reaction dynamics for $^3AQ^*$ quenched by antioxidant VC in EG homogeneous solution by using TR-EPR method combined with laser flash photolysis. The reaction intermediates and reaction

mechanism will be identified and analyzed. The rate constant for the quenching of excited triplet $^3AQ^*$ by VC will be measured.

2 Experimental

TR-EPR measurements were carried out at room temperature with a home-made X-band TR-EPR spectrometer, which has been described in detail elsewhere reported before [15]. The instrument consists of a conventional X-band EPR spectrometer (without field modulation), a boxcar integrator (Stanford SR 252), a digital oscilloscope (Philips PM 3350) and a broadband preamplifier with a 50 ns response time. Third-harmonic generation light (355 nm, 8 mJ) from a Nd: YAG laser (Spectrum Physics, INDI-40) operating at 20 Hz was used for photoexcitation. The microwave system adopts balance reflection bridge-type circuit and zero difference beat balanced frequency-mixing model. The gate width of the boxcar was fixed at 0.3 s and the sample of Boxcar integrator is 30. The sample solutions were deoxygenated by bubbling with N_2 for 20 minutes before each experiment. To avoid overheating, a peristaltic pump was used to circulate the sample solution through a flat quartz cell (optical path: 0.3 mm) in the EPR cavity.

The transient absorptive spectra were measured on the nanosecond laser flash photolysis setup at the Shanghai institute of applied physics, Chinese Academy of Sciences.

All chemicals used were analytically pure. Water was redistilled. AQ (Acros Organics) was purified by vacuum sublimation. VC (Acros Organics) is commercially available and used as received. The solvent was the mixture of EG and water (EG/H_2O=9/1, v/v). The concentration of AQ was 1mmol·L^{-1} in all solvent. Nitrogen was used to eliminate oxygen in the samples before CIDEP mesurement.

3 Results and discussion

3.1 Photoinduced reaction of AQ in EG-H_2O (EG/H_2O=9/1, v/v) solution

9,10-anthraquinone neutral radical AQH$^\bullet$ and ethylene glycol ketyl radical EG[-H]$^\bullet$ ($CH_2(OH)\dot{C}HOH$) have been observed in photolysis of AQ/EG system [13]. AQH$^\bullet$ and EG[-H]$^\bullet$ are produced through hydrogen atom transfer from solvent EG to $^3AQ^*$. When the saturated solution of AQ solved in a mixture solvent of EG-H_2O (EG/H_2O=9/1, v/v) was photolized, the CIDEP spectrum shown as Fig. 2 has been observed. It can be seen that the CIDEP spectrum in Fig. 2 still consists of two group hyperfine lines. The hyperfine lines in low field and high field shown as the stick spectrum (g=2.0041, $a_{H(\alpha)}$=1.75 mT, $a_{2H(\beta,CH2)}$=0.99 mT), $a_{H(\beta,OH)}$=0.1 mT can be assigned to ethylene glycol ketyl radical EG[-H]$^\bullet$ [16]. The broad peak in the center of spectrum, which is not well resolved, can be assigned to 9,10-anthraquinone neutral radical AQH$^\bullet$ [12,13]. AQH$^\bullet$ and EG[-H]$^\bullet$ were still believed to formed by a hydrogen atom transfer from solvent molecule EG to $^3AQ^*$.
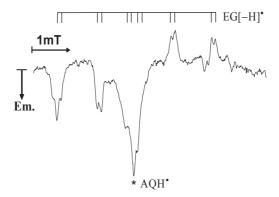
Figure 2: CIEDP spectrum observed during photolysis of AQ/EG-H$_2$O (EG/H$_2$O=9/1, v/v) solution, Delay: 0.6μs.

From the viewpoint of polarization pattern, the total CIDEP pattern in Fig. 2 is emissive (E), with a little E/A (low field side emission/high field side absorption) distortion. The E+E/A CIDEP pattern on each radical means the primary CIDEP mechanism is triplet mechanism (TM) [17-20], but there is a little contribution from the ST$_0$ mixing of the radical pair mechanism (ST$_0$-RPM) [19,20]. The dominant TM mechanism indicates the hydrogen atom transfer from EG to ^{3}AQ* occurs faster than the spin-lattice relaxation of ^{3}AQ*, and the spin polarization transfers effectively from parent ^{3}AQ* to the generated radicals AQH$^\bullet$ and EG[-H]$^\bullet$. So the primary photophysical and photochemical process during the photolysis of AQ in EG-H$_2$O (EG/H$_2$O=9/1, v/v) solution may be as following:

$$\tag{1}$$

Eq. 1 means the ground state of AQ reaches its single excited state ^{1}AQ* after 355 nm laser excitation, and then reaches its excited triplet ^{3}AQ* through intersystem crossing (ISC). Eq. 2 shows the relaxation of ^{3}AQ* and ^{3}T$_1$ is the spin-lattice relaxation time. Eq. 3 illustrates TM and ST$_0$-RPM polarization generation processes through ^{3}AQ* capturing hydrogen atom from EG, k_r is the hydrogen transfer reaction rate constant.

To make sure the assignment above is reasonable, the transient absorptive spectrum of photolysis of AQ in EG-H$_2$O (EG/H$_2$O=9/1, v/v) solution were measured under the condition of nitrogen saturation as shown in Fig. 3. It can be seen that there are two transient absorption peaks in Fig. 3, one is much strong with a maximum at 390 nm, the other is very weak at 490 nm. The stronger transient absorption peak at 390 nm can be attributed to ^{3}AQ*, and the weaker one at 490 nm can be attributed to AQH$^\bullet$

$$\text{(2)}$$

$$\text{(3)}$$

[21]. In addition, we can find that concurrent with the decrease of the absorption of $^3AQ^*$ at 390 nm is the little increase of the absorption of $AQH^\bullet$ at 490 nm which suggests there is a hydrogen transfer reaction from solvent EG to $^3AQ^*$ during the photolysis. So the assignment to the reaction intermediates and the analysis to the reaction mechanism during the photolysis are reliable.
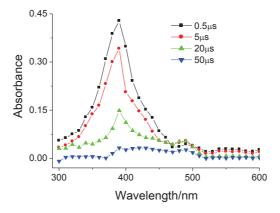

Figure 3: Transient absorptive spectrum observed during 355 nm laser flash photolysis of AQ in EG-H_2O (EG/H_2O=9/1, v/v) solution.

3.2 Photoinduced reaction of AQ and VC in EG-H$_2$O (EG/H$_2$O=9/1, v/v) solution

When solution of AQ and VC in EG-H_2O (EG/H_2O=9/1, v/v) is photolized, the CIDEP spectrum observed is shown in Fig. 4. Compared with Fig. 2, it can be seen that the signal of 9,10-anthraquinone neutral radical $AQH^\bullet$ and the ethylene glycol ketyl radical EG[-H]$^\bullet$ are still existing. However, there are two new strong hyperfine lines (shown as

the stick spectrum) on the low field side of the AQH$^{\bullet}$ signal which can be assigned to the VC monoanion radical AS$^{\bullet-}$(g=2.0054, a=0.19 mT) [22,23]. As we know, VC in the mixture of EG-H$_2$O (EG/H$_2$O=9/1, v/v) generally dissociate to VC monoanion A$_s$H$^-$ and proton H$^+$ [22]. So it is reasonable to believe that ^{3}AQ* may capture a hydrogen atom from A$_s$H$^-$ to form AQH$^{\bullet}$ and AS$^{\bullet-}$ (reaction Eq. (4)). Thus, ^{3}AQ* abstracts a hydrogen atom not only from EG but also from antioxidant VC during photolysis. There is a competition between hydrogen atom transfer from VC to ^{3}AQ* and that from EG to ^{3}AQ*.

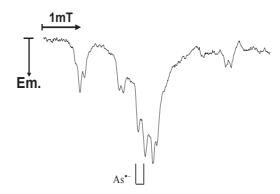Figure 4: CIDEP spectrum observed during photolysis of AQ and VC in EG-H$_2$O (EG/H$_2$O=9/1, v/v) solution, [VC]=20 mmol·L^{-1}, Delay: 0.6μs.

From the viewpoint of polarization pattern, the signal of AS$^{\bullet-}$ is emissive too. This means TM is the primary mechanism to generate CIDEP in the hydrogen atom transfer from A$_s$H$^-$ to ^{3}AQ*. The strong CIDEP signal of AS$^{\bullet-}$ means VC is an effective quencher for ^{3}AQ*.

$$\tag{4}$$

Based on the photophysical and photochemical reactions(1)-(4), the following quenching dynamics equation is obtained

$$\frac{I_0}{I} = 1 + k_q \tau [VC], \tag{5}$$

where

$$\tau = \frac{1}{^3T_1^{-1} + k_r}. \tag{6}$$

Here, I_0 and I represent the CIDEP intensity of EG[-H]$^{\bullet}$ in the absence of VC and pres-

ence of VC at concentration $[VC]$, respectively. τ represents the lifetime of $^3AQ^*$ in pure EG-H_2O(EG/H_2O=9/1, v/v) solution without VC. It can be seen from Eq. (5), I_0/I varies linearly with VC concentration $[VC]$. If we want to obtain the rate constant k_q, the slope for the variation of the I_0/I versus $[VC]$ and the lifetime τ must be obtained.
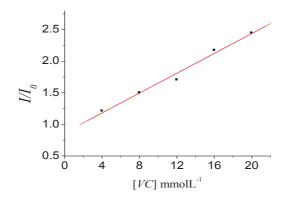
Figure 5: Quenching plot for $^3AQ^*$ by VC in EG-H_2O (EG/H_2O=9/1, v/v) solution without VC.

Fig. 5 is the quenching plot for $^3AQ^*$ by VC, the straightly fitted slope $k_q\tau$ is approximately 78.55 L·mol^{-1}. Fig. 6 is the transient absorbance decay curve of $^3AQ^*$ measured at 390 nm in EG-H_2O (EG/H_2O=9/1, v/v) solution without VC. From a single exponential fitting, the value of the lifetime τ is found to be approximately 12.3 μs. Since the slope $k_q\tau$ and lifetime τ have been determined, the quenching rate constant k_q can be obtained simply as about 0.639×10^7 L· mol^{-1}·s^{-1}.
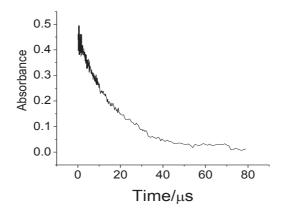
Figure 6: Decay curve of the transient absorbance of $^3AQ^*$ measured at 390nm in EG-H_2O (EG/H_2O=9/1, v/v) solution.

Here, the rate constants for the quenching of $^3AQ^*$ by VC in EG-H_2O (EG/H_2O=9/1, v/v) solution is of the order of 10^7 L· mol^{-1}·s^{-1}, while it is 10^9 L· mol^{-1}·s^{-1} order for excited triplet duroquinone ($^3DQ^*$) by 6-palmitoyl-L-ascorbic (PASCH$_2$) in ethanol and

acetonitrile [10]. It is well known that the photochemical reaction is nearly diffusion-controlled in solution with relatively larger viscosity. The rate of the diffusion-controlled reaction is related to solvent viscosity. The concrete relation between diffusion rate k_d and solvent viscosity η is:

$$k_d = \frac{8k_B T}{3\eta}. \tag{7}$$

where, k_B is the Boltzmann constant and T is the absolute temperature. From formula (7), we can see the diffusion rate constant k_d is inversely proportional to solvent viscosity η. As we know, the viscosity of ethanol and acetonitrile both are the order of 10^{-4}Pa·s[20,24]. While the viscosity of EG is 1.16×10^{-2}Pa·s[20], which is about 10^2 times larger than those of ethanol and acetonitrile. So, it is comprehensible that the rate constant for the quenching of ^{3}AQ* by VC here is of the order of 10^7L·mol^{-1}·s^{-1}. This result indicates the quenching reaction for ^{3}AQ* by VC in EG-H$_2$O (EG/H$_2$O=9/1, v/v) is also nearly diffusion-controlled from another point.

4 Conclusion

Time-resolved electronic paramagnetic resonance and laser flash photolysis techniques have been used to study the photoinduced reaction mechanism and dynamics for the quenching of ^{3}AQ* by antioxidant VC in EG-H$_2$O (EG/H$_2$O=9/1, v/v) solution. ^{3}AQ* abstracts hydrogen atom not only from solvent molecule EG but also from VC anion A$_s$H$^-$. VC is an effective quencher for ^{3}AQ*. ^{3}AQ* reacted with solvent EG and VC faster than the spin-lattice relaxation of ^{3}AQ*. The rate constant for the quenching ^{3}AQ* by VC is nearly diffusion-controlled. Accompanied by hydrogen atom transfer, there is spin polarization transfer from the parent molecule ^{3}AQ* to the generated radicals AQH$^\bullet$, EG[-H]$^\bullet$ and AS$^{\bullet-}$. These experimental results are helpful for understanding the mechanisms and dynamics for the quenching of photoininduced excited triplets of biological quinones by antioxidant VC.

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