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TR-ESR Investigation on Reaction of Vitamin C with Excited Triplet of 9,10-phenanthrenequinone in Reversed Micelle Solutions

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Time-resolved electron spin resonance has been used to study quenching reactions between the antioxidant Vitamin C (VC) and the triplet excited states of 9,10-phenanthrenequinone (PAQ) in ethylene glycol-water (EG-H_2O) homogeneous and inhomogeneous reversed micelle solutions. Reversed micelle solutions were used to be the models of physiological environment of biological cell and tissue. In PAQ/EG-H_2O homogeneous solution, the excited triplet of PAQ (^{3}PAQ*) abstracts hydrogen atom from solvent EG. In PAQ/VC/EG-H_2O solution, ^{3}PAQ* abstracts hydrogen atom not only from solvent EG but also from VC. The quenching rate constant of ^{3}PAQ* by VC is close to the diffusion-controlled value of 1.41×10^8 L/(mol·s). In hexadecyltrimethylammonium bromide (CTAB)/EG-H_2O and aerosol OT (AOT)/EG-H_2O reversed micelle solutions, ^{3}PAQ* and VC react around the water-oil interface of the reversed micelle. Exit of ^{3}PAQ* from the lipid phase slows down the quenching reaction. For Triton X-100 (TX-100)/EG-H_2O reversed micelle solution, PAQ and VC coexist inside the hydrophilic polyethylene glycol core, and the quenching rate constant of ^{3}PAQ* by VC is larger than those in AOT/EG-H_2O and CTAB/EG-H_2O reversed micelle solutions, even a little larger than that in EG-H_2O homogeneous solution. The strong emissive chemically induced dynamic electron polarization of $As^{\cdot-}$ resulted from the effective TM spin polarization transfer in hydrogen abstraction of ^{3}PAQ* from VC.

Key words: 9,10-phenanthrenequinone, Vitamin C, Time-resolved electron spin resonance, Reversed micelle

I. INTRODUCTION

9,10-phenanthrenequinone (PAQ) is one of the polycyclic quinine compounds in diesel exhaust. PAQ can be easily deposited in the respiratory tract and alveoli through respiration and enter biological cell and tissue through blood circulation. The excited triplet of PAQ (^{3}PAQ*) are formed by UV light irradiation in human skins and leaves of plants or by enzymatic and nonenzymatic redox cycling [1]. ^{3}PAQ* may produce damage to biological cell and tissue either directly by electron or hydrogen atom transfer from cellular proteins or indirectly by acting as sensitizers of singlet oxygen [2−5]. It has been reported that PAQ can improve reactive oxygen species (ROS) generation about 56.7% [4] and accelerate the cell apoptosis [5]. So the investigations on the quenching reaction of ^{3}PAQ* by biological antioxidant have been of great interests in recent years.

Vitamin C (ascorbic acid, VC, AsH_2) is a widely distributed, typical and well-known naturally available biological antioxidant. VC and Vitamin E (VE) have synergistic function in the biological antioxidation protection process [6−10]. In fact, VC can act directly as biological antioxidant to quench the oxidative excited triplet states of quinones. In sodium dodecyl sulfate (SDS) reversed micelle solution, VC can react with ^{3}DQ* (excited triplet of duroquinone), diffusing out from the micelle, through hydrogen atom transfer to make ^{3}DQ* deactivate [11]. In aerosol OT (AOT) reversed micelle solution, water-soluble VC and lipid-soluble $^3VK_3^*$ (excited triplet of Vitamin K_3) can react around the water-oil (w/o) interface region of reversed solution [12]. Nevertheless, there were no reports on the quantitive quenching dynamics of excited triplet states of quinones by VC up to now.

Time-resolved electron spin resonance (TR-ESR) is a suitable technique to detect and identify short-lived intermediate radicals directly in photo-induced reaction. The chemically induced dynamic electron polarization (CIDEP) signal observed by TR-ESR technique can provide a lot of information on photochemical reaction mechanism and reaction dynamics. In the present work, the reactions of photo-induced ^{3}PAQ* with antioxidant VC in ethylene glycol-water (EG-H_2O) homogeneous and inhomogeneous reversed micelle solutions were investigated by a TR-ESR technique. Transient absorptive spectrum was also used to measure the life-

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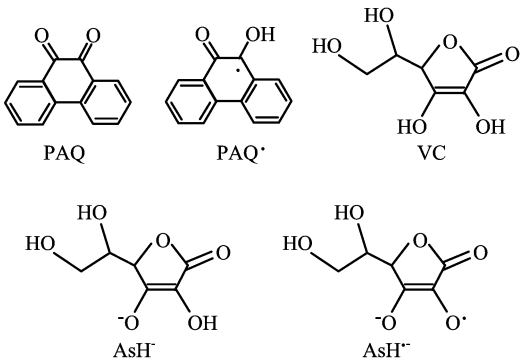

FIG. 1 Molecular structures of PAQ, PAQ·, VC, AsH^-, and $AsH^{\cdot -}$.

time of $^3PAQ^*$. The reversed micelle solutions were used to be the models of physiological environment of biological cell and tissue. The quenching mechanism and dynamics were analyzed in detail. The quenching rate constants of $^3PAQ^*$ by VC in various reversed micelle solutions were obtained. The influence of reaction microenvironment on the quenching mechanism and dynamics was discussed.

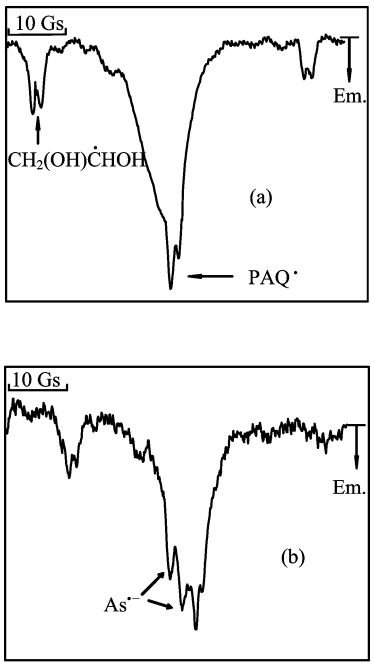

FIG. 2 CIDEP spectrum of photolysis of (a) PAQ/EG-H_2O and (b) PAQ/VC/EG-H_2O solution, C_{PAQ}=2 mmol/L, C_{VC}=20 mmol/L, T_d=0.6 μs.

II. EXPERIMENTS

TR-ESR measurements were performed on a homemade submicrosecond TR-ESR spectrometer without field modulation, which has been described in detail elsewhere [13, 14]. The instrument consists mainly of a conventional X-band ESR spectrometer, a boxcar integrator (Stanford SR 252), a digital oscilloscope (Philips PM 3350), and a broadband preamplifier with 50 ns time response. A K-129 klystron is used as microwave source. A Nd-YAG laser (Continuum Surelite11-10, THG355 nm) operating at the repetition of 20 Hz was used for photoexcitation. The gate width of the boxcar is 0.3 μs. The sample of boxcar integrator is 30. The sample solutions were deoxygenated by bubbling with N_2 before experiment, and was made to flow through a quartz flat cell (optical path: 0.3 mm) in the ESR cavity by a peristaltic pump.

The transient absorptive spectrum was measured on the transient absorptive spectrum setup in the lab of professor Li-min Zhang, University of Science and Technology of China. The excitation laser is also Nd-YAG 355 nm laser.

PAQ (Acros Organics, Fig.1) was carefully purified by vacuum sublimation. EG is of an A.R. grade. Water is redistilled water. Triton X-100 (TX-100, polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether, Acros Organics), aerosol OT (AOT, sodium bis(2-ethyl-1-hexyl) sulfosuccinate, Acros Organics), CTAB (hexadecyltrimethylammonium bromide, J&K Chemica) and VC (ascorbic acid, AsH_2, Acros Organics) were commercially available reagents and used as received. The mixed solvent is made up of EG and H_2O (EG:H_2O=9:1, volume ratio). The concentration of PAQ was 2 mmol/L. The concentration of TX-100, AOT, and CTAB in the EG-H_2O reversed micelle solution are all 0.5 mol/L.

III. RESULTS AND DISCUSSION

A. Reaction mechanism of photolysis of PAQ in homogeneous EG-H_2O solution

There is no CIDEP signal in photolysis of pure EG-H_2O solution. When PAQ/EG-H_2O solution is photolyzed, the CIDEP spectrum obtained at 0.6 μs delay is shown in Fig.2(a). The hyperfine coupling peaks in low field and high field (g=2.0041, $a_{H(\alpha)}$=1.7 mT, $a_{2H(\beta,CH_2)}$=0.93 mT, and $a_{H(\beta,OH)}$=0.95 mT) can be assigned to ethylene glycol ketyl radical $CH_2(OH)\dot{C}HOH$ [15, 16]. The other hyperfine peaks in the center of the spectrum can be assigned to neutral phenanthrenesemiquinone radical PAQH· (a_1=0.125 mT, a_2=0.026 mT). PAQH· and $CH_2(OH)\dot{C}HOH$ were produced by a hydrogen abstraction of $^3PAQ^*$ from solvent EG. The CIDEP spectrum is total emission with a slight E/A (low field side emission/high field side absorption) distortion. This suggests a superposition of the dominant net emissive polarization due to the triplet mechanism (TM) and a small E/A polarization due to radical pair mechanism (RPM).

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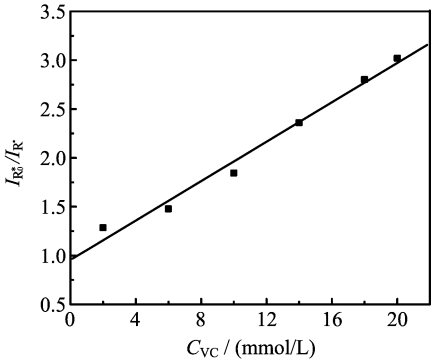

FIG. 3 Stern-Volmer plot of $I_{R_0^*}/I_{R\cdot}$ versus C_{VC} in photolysis of PAQ/VC/EG-H_2O solution.

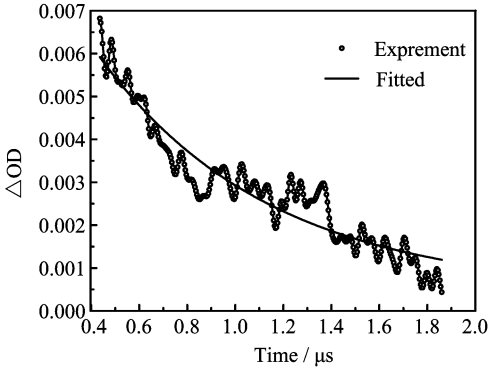

FIG. 4 Transient absorbance decay curve of ^{3}PAQ* in photolysis of PAQ/EG-H_2O solution.

When VC was added into PAQ/EG-H_2O solution, photolysis gave the CIDEP spectrum as shown in Fig.2(b). Compared to Fig.2(a), there are two new hyperfine lines in the center of the spectrum, which can be assigned to the ascorbate monoanion radical $As^{\cdot-}$ (g=2.0054, a=0.19 mT) [12, 17]. Formation process of $As^{\cdot-}$ can be comprehended as following: most of VC exist as the monoanion form (AsH^-, Fig.1) because VC releases a proton in aqueous solutions [17], then ^{3}PAQ* captures a hydrogen atom from AsH^- and $PAQH^{\cdot}$, $As^{\cdot-}$ were generated. So, ^{3}PAQ* can abstract a hydrogen atom not only from EG but also from VC in PAQ/VC/EG-H_2O solution. The total emission with a slight E/A distortion pattern CIDEP spectrum can also be explained by the superposition of TM and RPM contributions. So, the primary photophysical and photochemical process in the photolysis of PAQ/VC/EG-H_2O solution may be as follows,

$$\mathrm{PAQ} \xrightarrow{h\nu} {}^1\mathrm{PAQ}^* \xrightarrow{\mathrm{ISC}} {}^3\mathrm{PAQ}^* \tag{1}$$

$$^3\mathrm{PAQ}^* \xrightarrow{^3T_1^{-1}} \mathrm{PAQ} \tag{2}$$

$$^3\mathrm{PAQ}^* + \mathrm{CH_2(OH)CH_2OH} \xrightarrow{k_1} \mathrm{PAQ}^{\cdot} + \mathrm{CH_2(OH)\dot{C}HOH} \tag{3}$$

$$^3\mathrm{PAQ}^* + \mathrm{AsH}^- \xrightarrow{k_2} \mathrm{PAQ}^{\cdot} + \mathrm{AsH}^{\cdot-} \tag{4}$$

here, $^3T_1$ is the spin-lattice relaxation time of ^{3}PAQ*. k_1 is the reaction rate constant of ^{3}PAQ* with EG, and k_2 is the quenching rate constant of ^{3}PAQ* by VC.

B. Quenching dynamics of ^{3}PAQ* by VC in photolysis of PAQ/VC/EG-H_2O solution

Based on the reaction mechanism of Eqs.(1)−(4), the dynamics analysis can give the following Stern-Volmer equation,

$$\tau = \frac{1}{^3T_1^{-1} + k_1} \tag{5}$$

$$\begin{aligned}\frac{I_{R_0^*}}{I_{R\cdot}} &= 1 + \frac{k_2}{^3T_1^{-1} + k_1} C_{VC} \\ &= 1 + k_2\tau C_{VC}\end{aligned} \tag{6}$$

We make the following definition,

$$k_s = k_2\tau \tag{7}$$

In Eq.(5), $I_{R_0^*}$ and $I_{R\cdot}$ represent the CIDEP intensity of $CH_2(OH)\dot{C}HOH$ in the absence and presence of VC, respectively. τ is the lifetime of ^{3}PAQ* in pure EG-H_2O without VC. C_{VC} is VC concentration. From Eq.(5), it can be seen $I_{R_0^*}/I_{R\cdot}$ varies linearly with VC concentration and the slope is k_s. Eq.(6) tells us if we want to obtain the quenching rate constant k_2, the slope k_s of Stern-Volmer plot of $I_{R_0^*}/I_{R\cdot}$ versus C_{VC} and the lifetime τ of ^{3}PAQ* in EG-H_2O solution must be measured. With the $I_{R_0^*}/I_{R\cdot}$ measured in various C_{VC}, the Stern-Volmer plot of $I_{R_0^*}/I_{R\cdot}$ versus C_{VC} is fitted in Fig.3. We obtain k_s=100.88 L/mol.

In order to obtain the τ of ^{3}PAQ* in EG-H_2O solution, the following decay expression of ^{3}PAQ* can be obtained from Eqs. (2) and (3).

$$\begin{aligned}C_{^3\mathrm{PAQ}^*} &= C_{0^3\mathrm{PAQ}^*} \exp[-(^3T_1^{-1} + k_1)t] \\ &= C_{0^3\mathrm{PAQ}^*} \exp\left(\frac{t}{\tau}\right)\end{aligned} \tag{8}$$

here, $C_{^3\mathrm{PAQ}^*}$ is the initial concentration of ^{3}PAQ* after laser excitation. Figure 4 is the transient absorbance decay curve of ^{3}PAQ* at 680 nm and the fitted lifetime τ is 0.716 μs. Substitute k_s and τ into Eq.(7), the quenching rate constant k_2 of ^{3}PAQ* by VC is obtained as 1.41×10^8 L/(mol·s).

C. Quenching mechanism and dynamics of ^{3}PAQ* by VC in reversed micelle solution

Reversed micelle solutions are ideal models to simulate biological cell physiological environment *in vitro*

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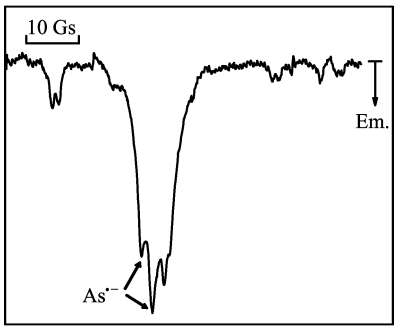

FIG. 5 CIDEP spectrum observed in photolysis of PAQ and VC in TX-100/EG-H_2O reversed micelle solution, T_d=0.6 μs, C_{VC}=10 mmol/L.

[18, 19]. In order to make our present experiments and the quenching reactions *in vivo* comparable, the photo-induced reaction of ^{3}PAQ* with VC in TX-100/EG-H_2O, AOT/EG-H_2O, and CTAB/EG-H_2O reversed micelle solutions were investigated.

Figure 5 shows the CIDEP spectrum observed at the delay time of 0.6 μs in the photolysis of PAQ and VC in TX-100/EG-H_2O reversed micelle solution. The CIDEP spectrum is an superposition of $CH_2(OH)\dot{C}HOH$, ascorbate monoanion radical $As^{\cdot-}$ and anion radical $PAQ^{\cdot-}$. $PAQ^{\cdot-}$ is produced from the dissociation of $PAQH^{\cdot}$, while $PAQH^{\cdot}$ is generated through hydrogen atom transfer reaction from EG, AsH^-, and TX-100 molecules to ^{3}PAQ*. As we know, TX-100 is non-anionic surfactant, most PAQ are solubilized inside the polyethylene glycol core [20]. So, ^{3}PAQ* can abstract directly hydrogen atom from TX-100 molecules. The high pH in polyethylene glycol core [21] results in the dissociation of $PAQH^{\cdot}$. This fact is consistent with the results on photolysis of p-benzoquinone (PBQ) [21] and naphthaquinone (NQ) [22] in TX-100 reversed micelle solution. On the other hand, the CIDEP signal intensities $As^{\cdot-}$ and $PAQ^{\cdot-}$ in Fig.5 are much stronger than those in homogeneous EG-H_2O solution (Fig.2(b)), although the VC concentration is only 10 mmol/L here. In AOT/EG-H_2O and CTAB/EG-H_2O reversed micelle solution, the CIDEP spectra (not shown) were the same as that in TX-100/EG-H_2O reversed micelle, but the total signal intensity is a little weaker.

The quenching rate constants of ^{3}PAQ* by VC in reversed micelle solutions were obtained with the same Stern-Volmer method (Table I). From Table I, it can be seen that the quenching rate constants of ^{3}PAQ* by VC are in the order of 10^7 or 10^8 L/(mol·s). They are 10−100 times less than that of ^{3}DQ* with $PASCH_2$ in ethanol and in acetonitrile [11]. It is well known that the diffusion controlled reaction rate is mainly determined by the diffusion rate of reactants in solution, and the diffusion coefficient satisfies the Stokes-Einstein relation

$$D = \frac{k_B T}{6\pi\eta r} \tag{9}$$

TABLE I Dynamic parameters of ^{3}PAQ* quenched by VC in homogeneous EG-H_2O solution and reversed micelle solutions. Units of k_s and k_q are L/mol and L/(mol·s).

System	k_s	τ/μs	k_q
PAQ/VC/EG-H_2O	100.88	0.716	1.41×10^8
PAQ/VC/AOT/EG-H_2O	84.01	1.898	4.43×10^7
PAQ/VC/CTAB/EG-H_2O	58.22	1.080	5.39×10^7
PAQ/VC/TX-100/EG-H_2O	74.49	0.485	1.53×10^8

where k_B is Boltzmann constant, η is solvent viscosity, and r is the radius of diffusive particle. In Eq.(9), diffusion coefficient D is inversely proportional to η. The viscosity of ethanol and acetonitrile are 0.997 [23] and 0.34 mPa·s [24] at room temperature, respectively. Nevertheless, the viscosity of EG is 11.55 mPa·s [23], and the viscosity of reversed micelle solution is much higher. So, it is comprehensible that the quenching rate constants of ^{3}PAQ* by VC in present work are in 10^7 or 10^8 L/(mol·s), close to be diffusion-controlled.

As we know, H_2O molecules are dispersed in the polar core to form a certain number of "water pools" in reversed micelle solution. Water-soluble VC are solubilized in the bulk water phase inside the micelle, while lipid-soluble PAQ exist in the oil phase outside the micelle. Thus, the reaction of ^{3}PAQ* with VC needs ^{3}PAQ* to diffuse out from the micelle and occur around the w/o interface region. This is the reason why the quenching rate constants of ^{3}PAQ* by VC in AOT/EG-H_2O and CTAB/EG-H_2O reversed micelle solution are smaller than that in homogeneous EG-H_2O solution.

An interesting phenomenon is that the quenching rate constant of ^{3}PAQ* by VC in TX-100/EG-H_2O reversed micelle solution is larger than those in homogeneous EG-H_2O and AOT/EG-H_2O, CTAB/EG-H_2O reversed micelle solution. As mentioned above, most PAQ are solubilized inside the hydrophilic polyethylene glycol core of TX-100/EG-H_2O reversed micelle, while water-soluble VC are also solubilized in the hydrophilic polyethylene glycol core. The high pH in this region [21] is good for the dissociation of VC to AsH^- and H^+. Photo-induced ^{3}PAQ* can abstract hydrogen atom from AsH^- directly. So, the quenching rate constant of ^{3}PAQ* by VC in TX-100/EG-H_2O reversed micelle solution is larger than those in AOT/EG-H_2O and CTAB/EG-H_2O reversed micelle solutions, even is larger than that in homogeneous EG-H_2O solution. On the other hand, the strong emissive CIDEP signal of $As^{\cdot-}$ (Fig.5) in the TX-100 reversed micelle solution, although the VC concentration is 10 mmol/L, suggests that ^{3}PAQ* reacted with AsH^- fast enough to transfer TM polarization before spin relaxation.

CTAB is one kind of cationic surfactant, while AOT is anionic surfactant. The "water pool" in CTAB/EG-H_2O reversed micelle solution is smaller than that in AOT/EG-H_2O reversed micelle solution [20, 25]. Widely dispersed small "water pool" improves the con-

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tact probability between $^3PAQ^*$ and AsH^-. On the other hand, the negative charge of the AOT micelle surface may keep AsH^- away from the micelle by the charge repulsion force. It is just these two factors mentioned above that make the quenching rate constants of $^3PAQ^*$ by VC in CTAB/EG-H_2O reversed micelle solution being a little larger than that in AOT/EG-H_2O reversed micelle solution.

IV. CONCLUSION

The quenching mechanism and dynamics of photoinduced $^3PAQ^*$ by VC in homogeneous and inhomogeneous reversed micelle solution were investigated by TR-ESR technique. Transient absorptive spectrum is used to measure the lifetime of $^3PAQ^*$. Photolysis of PAQ in EG-H_2O solution gave emissive CIDEP spectrum of $PAQH^{\cdot}$ and $CH_2(OH)\dot{C}HOH$, generated by hydrogen atom transfer reaction from EG to $^3PAQ^*$. When PAQ/VC/EG-H_2O solution was photolyzed, $^3PAQ^*$ abstract hydrogen atoms not only from EG but also from VC. The quenching rate constant of $^3PAQ^*$ by VC in EG-H_2O solution is 1.41×10^8 L/(mol·s). The reaction is close to be diffusion-controlled. During photolysis of PAQ and VC in CTAB/EG-H_2O and AOT/EG-H_2O reversed micelle solutions, lipid-soluble $^3PAQ^*$ and water-soluble VC react around the w/o interface of the reversed micelle. Exit of $^3PAQ^*$ from the lipid phase to w/o interface reduces the quenching rate constant of $^3PAQ^*$ by VC. For TX-100/EG-H_2O reversed micelle solution, PAQ and VC are solubilized in the same hydrophilic polyethylene glycol core. $^3PAQ^*$ abstract hydrogen atom from AsH^- and TX-100 fast enough in the coexisting region. The quenching rate constant of $^3PAQ^*$ by VC is larger than those in homogeneous EG-H_2O and inhomogeneous AOT/EG-H_2O, CTAB/EG-H_2O reversed micelle solution. The strong emissive CIDEP of $As^{\cdot-}$ resulted from the fast TM polarization transfer before $^3PAQ^*$ spin relaxation. These experimental results help us to comprehend further the biological antioxidation behavior of VC to excited triplet states of environment related quinones *in vivo*.

V. ACKNOWLEDGMENTS

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