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维生素 K₃ 的激发三重态与色氨酸、酪氨酸电子转移氧化反应的 激光闪光光解研究

唐睿智^{1,2} 李海霞^{1,2} 刘艳成^{1,2} 张 鹏^{1,2} 曹西艳^{1,3} 王文锋^{1,*}⁽¹中国科学院上海应用物理研究所, 上海 201800; ²中国科学院研究生院, 北京 100049;³湖南科技大学化学化工学院, 湖南 湘潭 411201)

摘要: 利用时间分辨的激光闪光光解技术研究了乙腈-水混合溶液(1:1, V/V)中 2-甲基萘醌(通常称为维生素 K₃)的激发三重态对色氨酸、酪氨酸的光敏氧化机理。通过瞬态吸收光谱的变化可以推断维生素 K₃ 的激发三重态可以与色氨酸、酪氨酸发生电子转移反应, 反应形成的维生素 K₃ 阴离子自由基的吸收峰可以直接从瞬态吸收谱图中观察到。维生素 K₃ 与色氨酸、酪氨酸的电子转移反应的速率分别为 1.1×10^9 和 0.6×10^9 L·mol⁻¹·s⁻¹。吉布斯自由能(ΔG)的计算结果表明维生素 K₃ 的激发三重态与色氨酸、酪氨酸电子转移反应在热力学上是可行的。

关键词: 维生素 K₃; 色氨酸; 酪氨酸; 激光闪光光解

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Laser Flash Photolysis Study on Electron Transfer Oxidation Reaction of Tryptophan or Tyrosine with Triplet State Vitamin K₃

TANG Rui-Zhi^{1,2} LI Hai-Xia^{1,2} LIU Yan-Cheng^{1,2} ZHANG Peng^{1,2}
CAO Xi-Yan^{1,3} WANG Wen-Feng^{1,*}⁽¹Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, P. R. China;²Graduate University of Chinese Academy of Sciences, Beijing 100049, P. R. China; ³School of Chemistry and Chemical Engineering, Hunan University of Science and Technology, Xiangtan 411201, Hunan Province, P. R. China)

Abstract: Laser flash photolysis was used to study the photosensitized oxidation mechanism of vitamin K₃, commonly known as 2-methyl-1,4-naphthoquinone (MQ), with tryptophan (TrpH) or tyrosine (TyrOH) in acetonitrile/water (1:1, V/V) solution. The triplet state of MQ reacted with TrpH or TyrOH by electron transfer with the formation of a MQ anion radical, which was directly observed in the transient absorption spectrum. The rate constants of the electron transfer reactions were determined to be 1.1×10^9 and 0.6×10^9 L·mol⁻¹·s⁻¹ for TrpH and TyrOH, respectively. The free energy changes (ΔG) of the reactions showed that the proposed electron transfer steps are thermodynamically feasible.

Key Words: Vitamin K₃; Tryptophan; Tyrosine; Laser flash photolysis

1 Introduction

The photosensitized oxidation of biomolecule by photo-oxidation reaction has received increased attention in recent years. The free radical theory of aging^{1,2} shows that aging is a result of destructive effects on biomolecule. Proteins are major poten-

tial targets for oxidation because they comprise approximately 68% of the dry mass of cells and tissues. Aromatic amino acid residues in proteins, especially tryptophan (TrpH) and tyrosine (TyrOH), are easily oxidized.^{3,4} Thus, the reactions of TrpH and TyrOH with photosensitizers have received considerable atten-

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*Corresponding author. Email: wangwenfeng@sinap.ac.cn; Tel: +86-21-39194602.

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tion in the field of proteins photo-oxidation.⁵⁻⁷ Oxidative damage to proteins occurs *via* complex chemical reactions that lead to changes in the proteins' structure and the rupture of the polypeptide chain as well as protein cross linking.⁸ The oxidative damage of proteins plays a critical role in the development of Alzheimer's disease, Parkinson's disease, cancer, and aging.⁹⁻¹²

Vitamin K₃, commonly known as 2-methyl-1,4-naphthoquinone (MQ), is one of the widely used endogenous photosensitizers with antihemorrhagic activity.¹³ MQ is present in vegetables and plays an active role in the photosynthetic mechanism. It also participates in cellular respiration as an electron transporter, and in oxidative phosphorylation¹⁴⁻¹⁷ as an effective electron carrier and electron transfer agent.^{18,19} Many studies have reported on the mechanisms of electron transfer oxidation of nucleotides by the triplet state of MQ (³MQ*) *via* laser flash photolysis technique.²⁰⁻²³ Charge transfer reactions between (³MQ*) and polyguanylic acid (polyG), guanine nucleotide (dGMP) demonstrated that polyG radical cation, dGMP radical cation, and MQ radical anion can be detected simultaneously. In recent years, Basu *et al.*²⁴⁻²⁶ has been concerning and studying magnetic field effects (MFEs) and medium-dependent effect on the photochemistry reactions between MQ and DNA, RNA bases, the results indicated that H atom transfer and electron transfer are the operative mechanisms depending upon the medium.

However, to our knowledge, compared with the research on the triplet state of MQ reacting with nucleosides, fewer studies have discussed the oxidation of proteins by the triplet state of MQ, owing to its complicated structure and mechanism. Therefore, it is a significant task to elucidate the mechanisms of interaction between the triplet state of MQ and protein monomer units or complexes. In the current study, TrpH and TyrOH are selected as models to observe the photo-oxidation mechanisms. We attempted to investigate the photo-processes of MQ with TrpH and TyrOH in acetonitrile/water (1:1, *V/V*) solutions using the 355 nm laser flash photolysis. The possible mechanisms are proposed and the related rate constants are obtained in this study.

2 Experimental

2.1 Materials

Vitamin K₃ (MQ), tryptophan (TrpH), tyrosine (TyrOH), and acetonitrile were purchased from Sigma-Aldrich. All the chemicals were analytically pure and used as received without further purification. All solutions were prepared freshly with pure water provided by Millipore purification system before each experiment. Samples were bubbled with high-purity N₂ or O₂ (99.999%) for 20 min before laser flash photolysis. All solutions were buffered with phosphate (1×10⁻³ mol·L⁻¹, pH 7.0).

2.2 Methods

Nd:YAG laser (NL303HT, EKSPLA, Lithuania) provided 355 nm pulse with duration of 5 ns and the maximum energy of 8 mJ per pulse was used as the pump light source. A xenon

lamp was employed as detecting light source. The laser beam and analyzing light passed perpendicularly through a quartz cell. The transmitted light entered a monochromator equipped with an R955 photomultiplier. The output signal from the HP54510B digital oscillograph was transferred to a personal computer for study. The laser flash photolysis setup has been previously described.²⁷

3 Results and discussion

3.1 Photoreaction of MQ with TrpH in acetonitrile/water (1:1) solution

As shown in Fig.1, in the transient absorption spectrum after 355 nm laser flash photolysis of the N₂-saturated acetonitrile/water (1:1) solution containing 0.1×10⁻³ mol·L⁻¹ MQ, a maximum absorption band at 370 nm was observed. The triplet state absorption of MQ (at 370 nm) decays by a first-order process with a rate of 3.4×10⁵ s⁻¹ in the N₂-saturated acetonitrile/water (1:1) solution containing 0.1×10⁻³ mol·L⁻¹ MQ. The maximum transient absorption at 370 nm can be quenched efficiently by O₂ (Fig.1 inset). This result was similar to the reported triplet state absorption spectrum of MQ.^{24,28}

Fig.2 shows the transient absorption spectra after 355 nm laser flash photolysis of the N₂-saturated acetonitrile/water (1:1) solution containing 0.1×10⁻³ mol·L⁻¹ MQ and 0.2×10⁻³ mol·L⁻¹ TrpH. Under such conditions, the transient absorption of the triplet state of MQ appeared immediately at the end of the laser flash pulse, and decayed significantly faster in the presence of TrpH. Following the decay of the triplet state of MQ at 370 nm, a spectrum with maximum absorption bands at 390 and 510 nm appeared. The energy of the triplet (*E_T*) of MQ is about 239 kJ·mol⁻¹,²⁹ while the *E_T* of TrpH is 297 kJ·mol⁻¹.³⁰ Therefore, the formation of the triplet state of TrpH *via* energy transfer from the triplet state of MQ is unlikely to occur. According to previous reports, the maximum absorption of the MQ anion radical²² and Trp[•]^{31,32} were located at 390 and 510 nm, respectively. Consequently, the transient absorption spec-

Fig.1 Transient absorption spectra recorded at (▲) 0.1 μs and (●) 1 μs after 355 nm laser photolysis of 0.1×10⁻³ mol·L⁻¹ MQ in N₂-saturated acetonitrile/water (1:1) solution

inset: transient time trace recorded at 370 nm in N₂-saturated (a), O₂-saturated (b) acetonitrile/water (1:1) solution

Fig.2 Transient absorption spectra recorded at (▲) 0.1 μs and (●) 2 μs after 355 nm laser photolysis of N_2 -saturated acetonitrile/water (1:1) solution containing $1 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ MQ and $2 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ TrpH

inset: transient kinetic traces recorded at (a) 390 nm and (b) 510 nm

tra resulting from MQ reacting with TrpH can be explained by electron transfer from TrpH to the triplet state of MQ, generating MQ anion radical and TrpH cation radical ($\text{TrpH}^{+\bullet}$). According to the pK_a given in the literature,⁷ the $\text{TrpH}^{+\bullet}$ ($\text{pK}_a=4.3$) turned into a neutral radical $\text{Trp}^\bullet$ by deprotonation under the experimental conditions. As shown in Fig.2 (inset), the formation of MQ anion radical and $\text{Trp}^\bullet$ radical was synchronous with the decay of the triplet state of MQ, which further demonstrated the electron transfer reaction between the triplet state of MQ and TrpH.

Varying the TrpH concentration (0.2×10^{-3} – $0.7 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$), the decay of $^3\text{MQ}^*$ was accelerated in the presence of TrpH, with rates proportional to the concentrations of TrpH (Fig.3), the rate constant of the reaction was determined to be $1.1 \times 10^9 \text{ L} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$ by monitoring the formation of MQ anion radical (Table 1). Thus, the mechanism of TrpH with the triplet state of MQ can be illustrated as the following:

3.2 Photoreaction of MQ with TyrOH in acetonitrile/water (1:1) solution

Fig.4 shows the transient absorption spectra after 355 nm laser flash photolysis of the N_2 -saturated acetonitrile/water (1:1) solution containing $0.1 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ MQ and $0.2 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$

Fig.3 Dependence of k_{obs} at 390 nm on the concentration of TrpH

Table 1 Rate constants and free energy changes (ΔG) of electron transfer from TrpH and TyrOH to the triplet state of MQ

Compound	$k/(\text{L} \cdot \text{mol}^{-1} \cdot \text{s}^{-1})$	E_{ox}/V	$\Delta G/(\text{kJ} \cdot \text{mol}^{-1})$
TrpH	1.1×10^9	1.01	−69.6
TyrOH	0.6×10^9	0.93	−79.2

L^{-1} TyrOH. Similar to the photoreaction of MQ with TrpH, the transient absorption of the triplet state of MQ appeared immediately after laser flash pulse and decayed significantly faster in the presence of TyrOH. However, after the decay of the triplet state of MQ, a spectrum with only a maximum absorption at around 390 nm formed. The transient absorbance of $\text{TyrOH}^{+\bullet}/\text{TyrO}^\bullet$ ³³ (at 410 nm) could not be observed clearly due to overlapping with that of the MQ anion radical or its lower absorption coefficient. The formation of the MQ anion radical was also observed to be synchronous with the decay of the triplet state of MQ (Fig.4 inset). The formation of triplet state of TyrOH via energy transfer from triplet state of MQ is also unlikely to occur because the E_T of TyrOH ($342 \text{ kJ} \cdot \text{mol}^{-1}$)³⁰ is higher than that of MQ ($239 \text{ kJ} \cdot \text{mol}^{-1}$). Therefore, It is concluded that the electron transfer from TyrOH to the triplet state of MQ also occurs. The rate constant of MQ with TyrOH was acquired in a manner similar to the photoreaction of MQ with TrpH (Fig.5), the rate constant of the reaction was determined to be $0.6 \times 10^9 \text{ L} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$. The reaction mechanism of TyrOH with the triplet state of MQ can be illustrated as the following:

3.3 Calculation of the free energy changes (ΔG) for the electron transfer reactions

To further prove the thermodynamic feasibility of the reaction, the free energy changes (ΔG) of the electron transfer between the triplet state of MQ and TrpH or TyrOH can be calculated by the Rehm-Weller equation:³⁴

$$\Delta G = 96.48(E_{\text{ox}} - E_{\text{red}} - e^2/\epsilon d) - E_{0,0}$$

where E_{ox} (in V) and E_{red} (in V) are the oxidation potentials of the donor and the reduction potential of the acceptor, respec-

Fig.4 Transient absorption spectra recorded at (▲) 0.1 μs and (●) 3 μs after 355 nm laser photolysis of N_2 -saturated acetonitrile/water (1:1) solution containing $0.1 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ MQ and $0.2 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ TyrOH

inset: transient kinetic trace recorded at 390 nm

Fig.5 Dependence of k_{obs} at 390 nm on the concentration of TyrOH

tively, and $E_{0,0}$ (in $\text{kJ} \cdot \text{mol}^{-1}$) is the triplet state energy of MQ. Here, $e^2/\epsilon d$ is the coulombic term, equal to 0.024 eV in acetonitrile/water (1:1) solution.³⁵ The E_{red} and $E_{0,0}$ values of MQ are -0.77 V (vs saturated calomel electrode (SCE)) and 239.3 $\text{kJ} \cdot \text{mol}^{-1}$, respectively.²⁹ The E_{ox} values of TrpH and TyrOH are 1.01 and 0.93 V (vs normal hydrogen electrode (NHE)), respectively.³⁶ Therefore, the ΔG values obtained for TrpH and TyrOH are -69.6 and -79.2 $\text{kJ} \cdot \text{mol}^{-1}$, respectively (Table 1). Thus, the photo-induced electron transfer from TrpH and TyrOH to the triplet state of MQ is thermodynamically favorable.

4 Conclusions

The above experimental results indicated that the triplet state of MQ reacted with TrpH and TyrOH by electron transfer with the formation of MQ anion radical or oxidized radicals of Trp $^{\bullet}$ and TyrO $^{\bullet}$. The rate constants were determined to be 1.1×10^9 and 0.6×10^9 $\text{L} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$ for TrpH and TyrOH, respectively, and the free energy changes show that the electron transfer reactions are thermodynamically feasible in our experiment. Knowledge of the electron transfer oxidation of TrpH and TyrOH by the triplet state of MQ is important to better understand structural effects on the photochemical behavior of proteins with the triplet state of MQ.

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