

Photosensitized oxidation of tryptophan and tyrosine by aromatic ketones: A laser flash photolysis study

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Photochemical processes of benzophenone (BP) and xanthone (XT) with tryptophan (TrpH) and tyrosine (TyrOH) were studied using the laser flash photolysis technique. It has been observed that the triplet state of BP and XT reacted with TrpH and TyrOH by hydrogen transfer with the formation of BP and XT ketyl radicals and oxidized radicals of Trp[•] and TyrO[•]. The related rate constants of these reactions were determined in this paper. The free energy changes (ΔG) of these reactions suggested that the proposed hydrogen transfer mechanism was thermodynamically feasible. These results provide theoretical foundation for further studying structural effects on the photochemical behaviors of proteins with triplet state BP and XT.

benzophenone, xanthone, tryptophan, tyrosine, laser flash photolysis

1 Introduction

The photochemistry of aromatic ketones especially benzophenone (BP) and xanthone (XT) has been extensively studied because they are widely used as photosensitizers as well as electron or hydrogen atom acceptors [1, 2]. At present, three kinds of aromatic ketones triplet excited states ($n-\pi^*$, $\pi-\pi^*$, CT) have been identified, which are responsible for different photophysical and photochemical reactions [3, 4]. The triplet state of aromatic ketones can undergo two main ways: either by direct transfer of hydrogen atoms or by electron transfer from a variety of substrates including aliphatic hydrocarbons, alcohols, phenols, and amines to the aromatic ketones. The reactivity of aromatic ketones triplet state ($n-\pi^*$) is higher in hydrogen abstraction than that of the triplet state ($\pi-\pi^*$) in non-polar solvents. The property of the triplet state of aromatic ketones depends on the substituent and solvent polarity. However, there is no fixed

ratio of the reactivity of $n-\pi^*$ and $\pi-\pi^*$ triplets [5].

Electron transfer and hydrogen transfer reactions are closely interrelated to not only chemistry but also biochemistry. Proteins are the major potential targets for oxidation because they comprise approximately 68% of the dry weight of cells and tissues. Aromatic amino acid residues in proteins, especially tryptophan (TrpH) and tyrosine (TyrOH), are easily oxidized because they have the strongest absorption in near UV [6, 7]. Oxidative damage to proteins via complex chemical reactions leads to the changes of proteins' structural, conformation, fragmentation and rupture of the polypeptide chain as well as proteins' crosslinking [8]. Convincing evidence has indicated that oxidative damage of proteins plays a critical role in the development of Alzheimer's disease, Parkinson's disease, cancer, and aging [9, 10].

In this paper, we attempt to investigate the photoprocesses of BP, XT and their photoreactions with TrpH, TyrOH in acetonitrile/water (1:1, v/v) solution using 355 nm laser flash photolysis. The possible mechanisms are proposed and the related rate constants are obtained.

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2 Materials and methods

2.1 Materials

Benzophenone (BP), xanthone (XT), tryptophan (TrpH), tyrosine (TyrOH) and acetonitrile were purchased from J&K Chemical Ltd. and used 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. The solutions were buffered with phosphate (1 mM, pH 7.0).

2.2 Methods

Nd: YAG laser provides 355 nm pulses with duration of 5 ns and the maximum energy of 80 mJ per pulse was used as the pump light source. A Xenon lamp was employed as the 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.

3 Results and discussion

3.1 Transient absorption spectra of BP and XT in acetonitrile/water (1:1, v/v) solution

Figures 1 and 2 show the transient absorption spectra after the 355 nm laser flash photolysis of N₂-saturated solution containing 0.2×10^{-3} mol L⁻¹ BP and 0.05×10^{-3} mol L⁻¹ XT, respectively. The spectra recorded 0.1 and 5 μs after the laser flash photolysis were characterized by two strong absorption bands with maximum at 320 and 520 nm (Figure 1) and a maximum absorption band at 600 nm (Figure 2), respectively, which can be quenched efficiently by O₂ (Figure 1 insert and Figure 2 insert). The bands were similar to the reported characteristic triplet state absorption of BP (³BP*) [11, 12] and XT (³XT*) [13, 14]. Furthermore, a bleaching was observed in the XT solution at 340 nm. Such bleaching was contributed by the strong absorption of ground-state XT.

3.2 Photoreactions of BP with TrpH, TyrOH in acetonitrile/water (1:1, v/v) solution

Figure 3 shows the transient absorption spectra after the laser flash photolysis of N₂-saturated solution containing 0.2×10^{-3} mol L⁻¹ mM BP and 0.4×10^{-3} mol L⁻¹ mM TrpH. It is observed from the figure that the transient absorption of the triplet state of BP appeared instantly after the laser flash photolysis. In the presence of TrpH, following the decay of the triplet state of BP, a new spectrum with maximum ab-

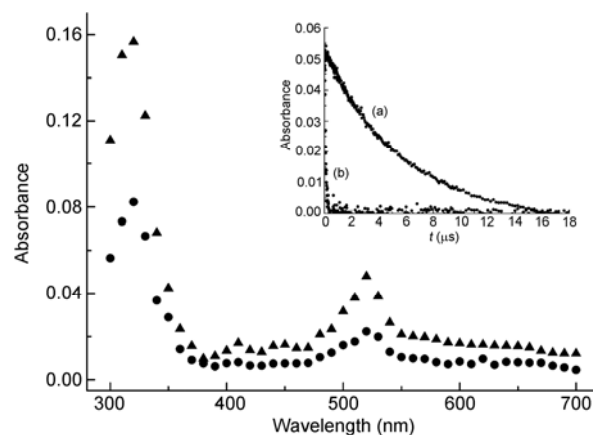


Figure 1 Transient absorption spectra recorded at 0.1 μs (▲) and 5 μs (●) after 355 nm laser flash photolysis of N₂-saturated solution: (a) 0.2×10^{-3} mol L⁻¹ BP. Insert: Transient time traces measured at 520 nm in N₂-saturated (a) and O₂-saturated (b) solution.

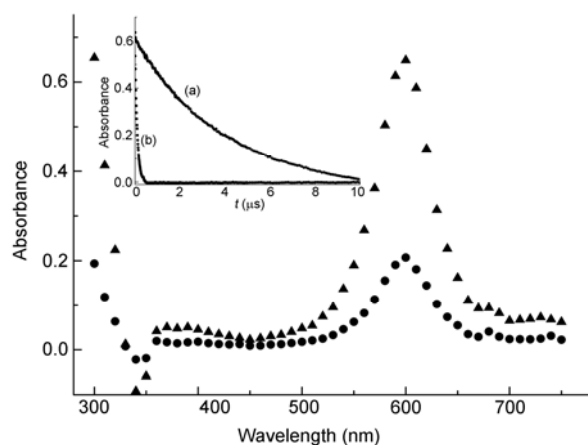


Figure 2 Transient absorption spectra recorded at 0.1 μs (▲) and 5 μs (●) after 355 nm laser flash photolysis of N₂-saturated solution 0.05×10^{-3} mol L⁻¹ mM XT. Insert: Transient time traces measured at 600 nm in N₂-saturated (a) and O₂-saturated (b) solution.

sorptions at around 330 and 540 nm appeared. According to a previous study, the maximum absorptions of BP ketyl radical and Trp• radical are located at 330, 540 and 510 nm [15, 16], respectively. The transient absorption of Trp• at 510 nm was not observed clearly due to its overlapping with that of the BP ketyl radical absorption or its lower absorption coefficient. The reaction of the triplet state with TrpH may be primarily controlled by (n-π*) because no apparent BP anion radical absorption at 630 nm was observed [17]. Therefore, we conclude that the hydrogen transfer from TrpH to triplet-state BP generated the BP ketyl and Trp•. As shown in Figure 3 (insert), the formation of the BP ketyl radical was synchronous with the decay of triplet-state BP, which further demonstrated that the reaction between triplet-state BP and TrpH occurred.

Figure 4 shows the transient absorption spectra after the laser photolysis of N₂-saturated solution containing $0.2 \times$

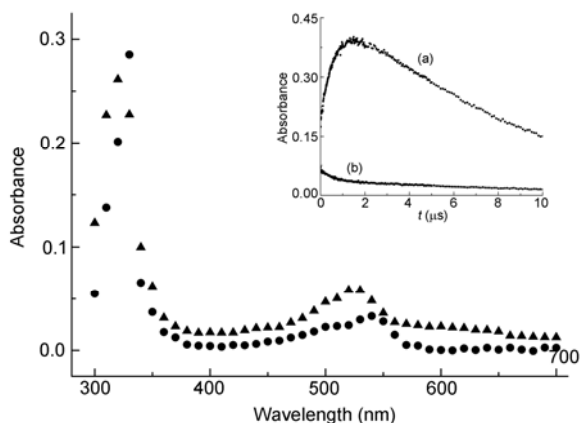


Figure 3 Transient absorption spectra recorded at 0.1 μs (▲) and 5 μs (●) after 355 nm laser photolysis of N₂-saturated aqueous solution containing 0.2×10^{-3} mol L⁻¹ BP and 0.4×10^{-3} mol L⁻¹ mM TrpH. Insert: Transient time traces measured at 330 (a) and 520 nm (b).

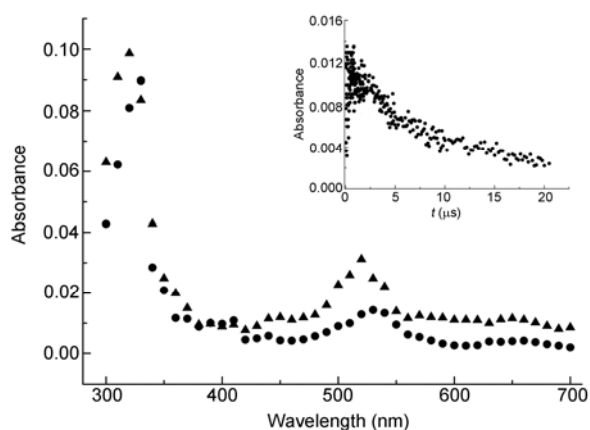
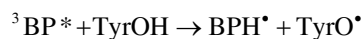
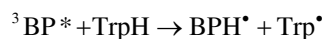


Figure 4 Transient absorption spectra recorded at 0.1 μs (▲) and 4 μs (●) after 355 nm laser photolysis of N₂-saturated aqueous solution containing 0.2×10^{-3} mol L⁻¹ BP and 0.4×10^{-3} mol L⁻¹ TyrOH. Insert: Transient time traces measured at 410 nm.

10^{-3} mol L⁻¹ BP and 0.4×10^{-3} mol L⁻¹ TyrOH. Similar to the photoreaction of BP with TrpH, the triplet state absorption of BP appeared immediately after the laser pulse, accompanying the decay of the triplet state absorption of BP, and three new absorption peaks at 330, 410 and 540 nm appeared. The absorption peaks at 330 and 540 nm can also be attributed to the BP ketyl radical, and another absorption peak at 410 nm belonged to TyrO• [18]. The formation of TyrO• is shown in Figure 2 (insert). The result also indicated that hydrogen transfer from TyrOH to the triplet state of BP occurred, and led to the formation of the BP ketyl radical and TyrO•.

After varying the TrpH and TyrOH concentrations (0.1×10^{-3} mol L⁻¹ – 0.6×10^{-3} mol L⁻¹), the rate constants of the reactions were determined to be 1.1×10^{10} and 0.8×10^{10} L mol⁻¹ s⁻¹ by monitoring the formation of the BP ketyl radical (Table 1). Therefore, the mechanisms of TrpH and

TyrOH with the triplet state of BP are illustrated as



3.3 Photoreactions of XT with TrpH, TyrOH in acetonitrile/water (1:1, v/v) solution

Similar experiments were performed by using TrpH and TyrOH as the quenchers of the triplet state of XT. Figure 5 shows the transient absorption spectra after the 355 nm laser flash photolysis of N₂-saturated solution containing 0.05×10^{-3} mol L⁻¹ XT and 0.2×10^{-3} mol L⁻¹ TrpH. The triplet state of XT (³XT*) immediately appeared after the laser flash pulse. In the presence of TrpH, the absorptions at 340 and 500 nm grew on the same time scale as the disappearance of the triplet state of XT. This phenomenon was similar to a previously reported disappearance of the XT ketyl radical [19]. However, the transient absorbance of Trp• was not observed clearly due to its overlapping with the XT ketyl radical. Moreover, the absorption of the XT anion radical was not observed probably because the triplet-state XT reacting with TrpH belonged to (n-π*). Figure 5 (insert) shows the simultaneous triplet-state XT decay and XT ketyl radical formation. This finding demonstrated the occurrence of the reaction between triplet-state XT and TrpH.

A similar experiment was performed by using TyrOH as the quencher of ³XT*. After the laser flash photolysis of XT in the presence of TyrOH, two apparent transient absorption bands located at 340 and 410 nm were formed (Figure 6). In the presence of TyrOH, similar to the BP reaction with TrpH, TyrO• and XT ketyl radicals were formed. The kinetic trace observed at 410 nm is shown in Figure 6 (insert). Therefore, hydrogen was transferred from TyrOH to

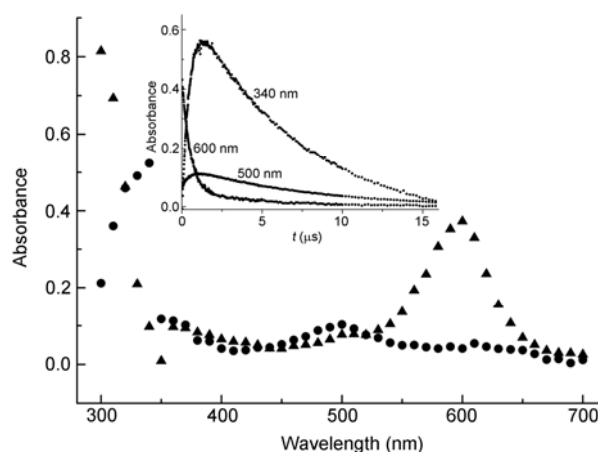


Figure 5 Transient absorption spectra recorded at 0.1 μs (▲) and 3 μs (●) after 355 nm laser photolysis of N₂-saturated aqueous solution containing 0.05×10^{-3} mol L⁻¹ XT and 0.2×10^{-3} mol L⁻¹ TrpH. Insert: Transient time traces measured at 340, 600 and 500 nm.

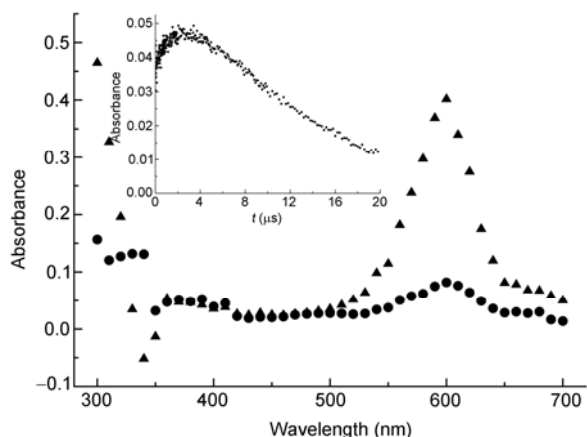
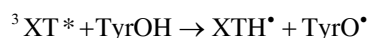
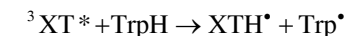


Figure 6 Transient absorption spectra recorded at 0.1 μs (▲) and 1 μs (●) after 355 nm laser photolysis of N_2 -saturated aqueous solution containing $0.05 \times 10^{-3} \text{ mol L}^{-1}$ XT and $0.2 \times 10^{-3} \text{ mol L}^{-1}$ TyrOH. Insert: Transient time traces measured at 600 nm.

$^3\text{XT}^*$, and the XT ketyl as well as TyrO^\bullet radicals were formed.

The rate constants of the reactions were determined according to the formation of the BP ketyl radical at 330 nm and the decay of $^3\text{XT}^*$ at 600 nm (Table 1). The photo-oxidation processes of TrpH and TyrOH with $^3\text{XT}^*$ are then described as follows:



4 Calculation of free energy changes (ΔG) for the hydrogen transfer reactions

The above experiments suggest that the triplet state of BP and XT react with TrpH, TyrOH by hydrogen transfer with the formation of BP ketyl radical, XT ketyl radical and oxidized radicals of Trp^\bullet , TyrO^\bullet . To further demonstrate the thermodynamic feasibility, free energy changes (ΔG) for electron transfer between triplet state of BP, XT and TrpH, TyrOH are calculated by the Rehm–Weller equation [20].

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

where E_{ox} (V) and E_{red} (V) are the oxidation potential of the donor and the reduction potential of the acceptor, respectively, and $E_{0,0}$ (kJ/mol) is the triplet state energy of MQ. Here $e^2/\epsilon d$ is the Coulombic term and in acetonitrile/water (1:1, v/v) solution it is 0.024 eV [21]. The E_{red} and $\Delta E_{0,0}$ values of BP and XT are -1.589 V [22] (vs. NHE), -1.519 V [23] (vs. NHE) and 289 kJ/mol [24], 309.8 kJ/mol [25], respectively. The E_{ox} values of TrpH and TyrOH are 1.01 V (vs. NHE) and 0.93 V (vs. NHE) [26]. Therefore, the ΔG values are obtained as shown in Table 1. Thus, the photo-induced hydrogen transfer from tryptophan (TrpH), tyro-

Table 1 Rate constants and free energy changes (ΔG) of hydrogen transfer from TrpH and TyrOH to triplet state BP and XT

Substrate	Benzophenone		Xanthone	
	$k (\text{L mol}^{-1} \text{s}^{-1})$	$\Delta G (\text{kJ mol}^{-1})$	$k (\text{L mol}^{-1} \text{s}^{-1})$	$\Delta G (\text{kJ mol}^{-1})$
TrpH	1.1×10^{10}	−40.6	2.0×10^{10}	−68.1
TyrOH	0.8×10^{10}	−48.3	1.5×10^{10}	−77.8

sine (TyrOH) to the triplet state of BP and XT is thermodynamically favored.

5 Conclusions

Overall, the results indicated that TrpH and TyrOH underwent hydrogen transfer with the lowest-lying triplet state ($n-\pi^*$) of BP and XT via the time-resolved technique of laser flash photolysis. Consequently, BP and XT ketyl radicals, as well as Trp^\bullet and TyrO^\bullet radicals were formed. The rate constants and free energy changes (ΔG) of the hydrogen transfer reactions were calculated. The hydrogen transfer oxidation processes of TrpH and TyrOH by triplet-state BP and XT are important for better understanding structural effects on the photochemical behaviors of proteins with BP and XT.

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