

A LASER FLASH PHOTOLYSIS STUDY OF AMINO ACIDS AND DIPEPTIDES USING 4-NITROQUINOLINE 1-OXIDE AS A PHOTSENSITIZER: THE pH DEPENDENCE

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Abstract--The pH effects on the photochemical reaction of amino acids and related dipeptides with 4-nitroquinoline 1-oxide (4NQO) as a photosensitizer have been investigated by laser flash photolysis. The obtained kinetic parameters show that the electron transfer from Tryptophan (Trp), Tyrosine (Tyr) as well as dipeptides containing Trp and/or Tyr residue to triplet 4NQO (^T4NQO) are efficient, but inefficient from methionine (Met) and dipeptides containing neither Trp nor Tyr. The result was supported by the calculated values of the free energy change from measured oxidation potentials for the electron transfer. It was demonstrated that Trp and Tyr residues are initial reaction sites with ^T4NQO, while Tyr/O[•] radical may be final species for Trp-Tyr dipeptide. In acidic aqueous solutions, the self-quenching rate constants of ^T4NQO and the rate constants of electron transfer from amino acids to ^T4NQO decrease with decreasing pH. In alkaline solutions, amino acids are easily oxidized by 4NQO under irradiation of laser pulse, and no transient absorption signal was observed.

INTRODUCTION

4-Nitroquinoline 1-oxide (4NQO) is one of the typical carcinogenic compounds. This compound has the ability to bind to DNA via charge-transfer interaction in aqueous solutions [1,2]. A number of papers have been published on its chemical properties and biological effects [1-9].

Photochemical reactions of 4NQO with amino acids, DNA and related compounds in aqueous solutions have been studied by using laser flash photolysis technique [6,7]. Under irradiation with a 355 nm light pulse, the triplet state of 4NQO (^T4NQO) formed, which was characterized by an absorption spectrum with maxima at 410 and 590 nm. The quenching rate constants of triplet 4NQO have been obtained by measuring the absorption decay of triplet 4NQO, and the electron transfer

mechanism from quenchers to triplet 4NQO has been demonstrated. Arai *et al.* found that the compounds possessing lower oxidation potentials, Tryptophan (Trp), Tyrosine (Tyr) and proteins containing Trp or/and Tyr residues can efficiently quench triplet 4NQO at nearly diffusion-controlled rate, but other amino acids and proteins containing neither Trp nor Tyr residue have lower quenching rate constants as $\sim 10^7 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$.

In this work, we investigated the self-quenching of triplet 4NQO, photochemical reactions of 4NQO with amino acids and their pH effects. It is significant to understand the interactions of 4NQO and protein in different pH values. Moreover, the photoinduced electron transfer from some dipeptides to 4NQO has also been studied.

MATERIALS AND METHODS

A conventional laser flash photolysis technique was used in the present experiment. The apparatus and procedures used in this paper have been described elsewhere [10]. Excitation light was the third harmonic (355 nm) from a Nd:YAG laser (Max-Laser Kids of ICT Lasers Ltd.) with a duration of 10 ns. The source of analytical light was a 500W Xenon lamp. The laser and analytical light beam passed perpendicularly through a quartz cell with an optical path length of 10 mm. The analyzing light beam, after once passing the cell, entered a monochromat (MC-30N of Ritsu Oyo Kogaku Co. Ltd.) equipped with a GDB59 photomultiplier at the rear of the exit slit. The signals from the photomultiplier were displayed as a function of time on a Tektronix TDS 380 oscilloscope (average 256 times). Transient spectra were determined from a series of oscilloscope traces obtained with the same solution in a point-by-point manner with respect to wavelengths.

Absorption and fluorescence spectra were measured on UV-VIS (Shimadzu UV-2100) and fluorescence (970 CRT) spectrophotometers respectively. Redox potentials were measured by cyclic voltammetry (CV-27).

4NQO was purchased from Sigma Chemical Co. All amino acids and dipeptides were also obtained from Sigma Chemical Co.

Solutions were prepared by using triply distilled water. In our experiment, the pH values of solutions were adjusted by NaOH and H_3PO_4 (or HClO_4) solutions. All samples were deaerated by high purity nitrogen (99.99%) bubbling for 20 min. All experiments were carried out at room temperature.

RESULTS AND DISCUSSION

Photophysical Process of 4NQO and Its pH Effect

4NQO has two absorption bands with maxima at 249nm and 363nm (see Figure 1a), which are assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively. Fluorescence spectrum of 4NQO shows a weak emission with $\lambda_{\max} = 484.3$ nm (Figure 1b), which corresponds to the lowest energy level of singlet excited state of 4NQO.

As shown in Figure 2, transient absorption spectra were observed from laser photolysis of 0.25×10^{-3} M 4NQO aqueous solution at pH 7.0. At $5 \mu\text{s}$ after the laser pulse, a transient absorption spectrum with maximum at 410 and 590 nm appears resulting from absorption of triplet excited state of 4NQO (^T4NQO) [6,7]. The two absorption peaks disappear at $20 \mu\text{s}$, and a slow-decaying spectrum with absorption

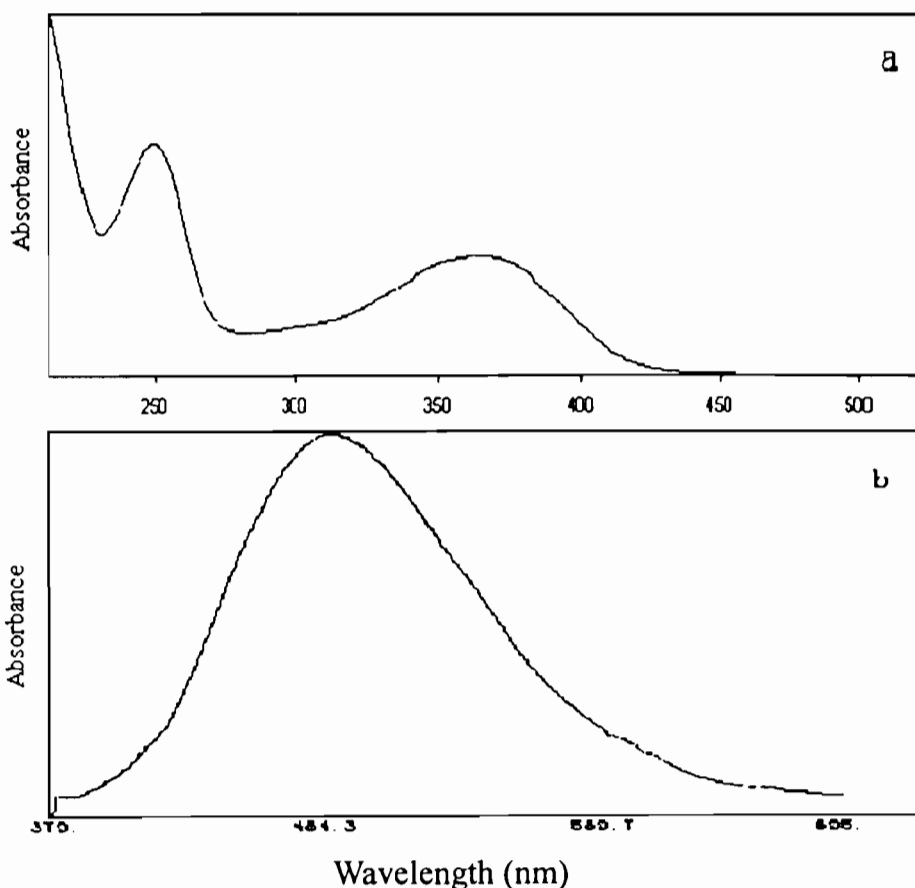


Figure 1. UV-visible (a) and Fluorescence (b) spectra of 4NQO (0.1×10^{-3} mol dm^{-3}) in aqueous phosphate buffer (pH = 7.0).

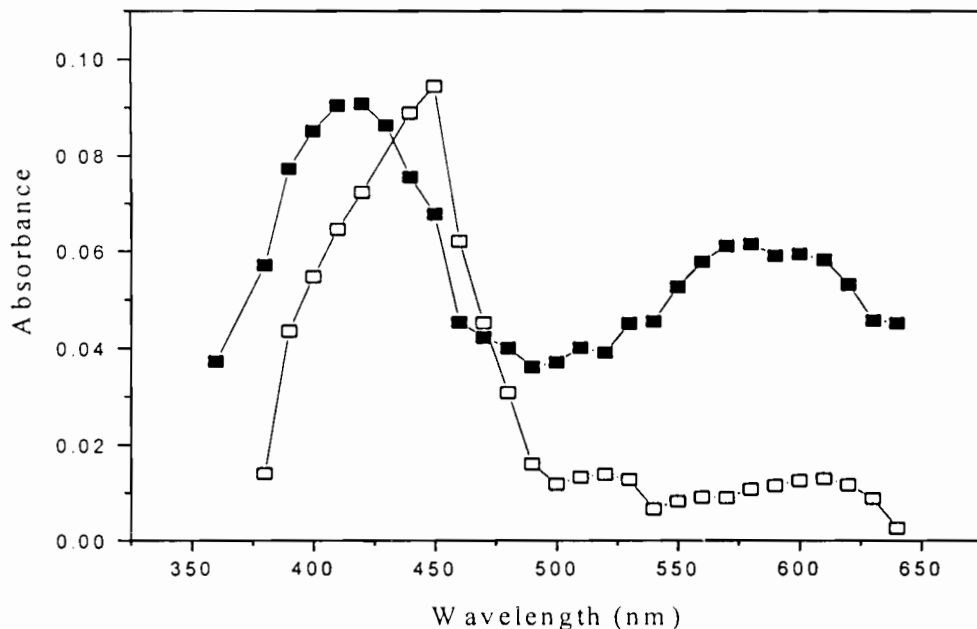
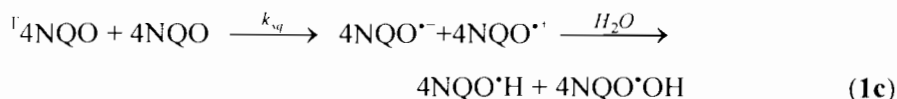
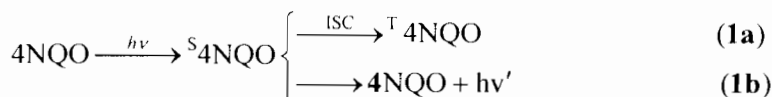


Figure 2. Transient absorption spectra obtained in neutral phosphate buffer solution of 4NQO ($0.25 \times 10^{-3} \text{ mol dm}^{-3}$). $5 \mu\text{s}$ (■) and $20 \mu\text{s}$ (□) after laser pulse.

maximum at 450nm appears subsequently. The slow-decaying species could be assigned to $4\text{NQO}^*\text{H}$ [6] and $4\text{NQO}^*\text{OH}$. From these results, the photophysical and possible photochemical processes of 4NQO can be depicted as follows:



The triplet state of 4NQO decays according to pseudo-first-order kinetics. Its decay rate constant $1/\tau_T$ can be expressed as [11,12]:

$$1/\tau_T = 1/\tau_T^0 + k_{sq}[4\text{NQO}] \quad \text{(2)}$$

in which τ_T^0 represents the intrinsic triplet lifetime of ${}^T4\text{NQO}$.

The quenching rate of triplet state of 4NQO can be obtained from the absorption decay at 590 nm (Figure 3). The decay trace of ${}^T4\text{NQO}$ follows first-order kinetics as shown in the inset of Figure 3. The slope is the decay rate constant $k_{\text{obs}}(1/\tau_T)$ of ${}^T4\text{NQO}$. The plot of k_{obs} against the concentration of 4NQO is a straight line as shown in Figure 4.

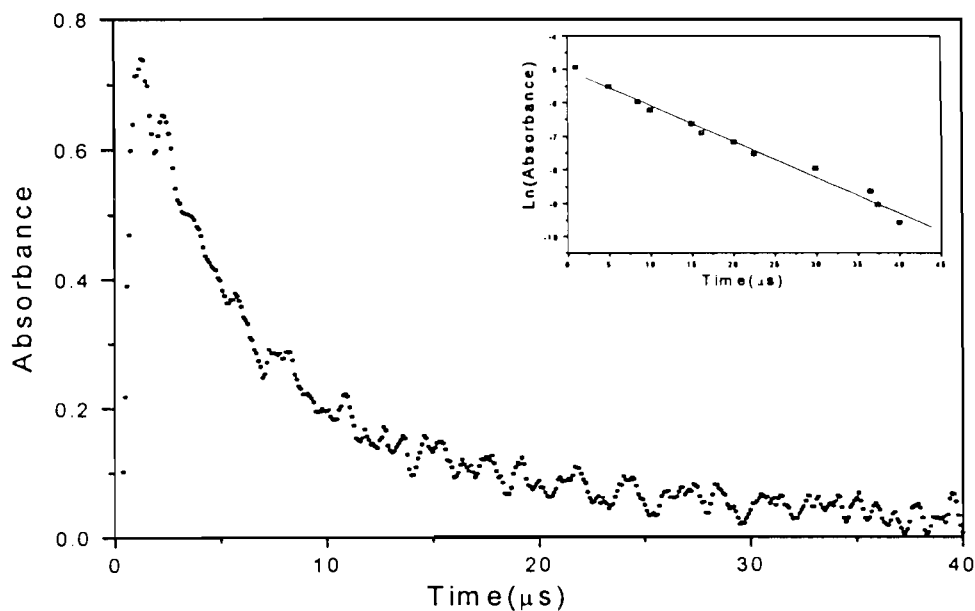


Figure 3. Transient absorption decay trace obtained by photolysis of the aqueous solution of 4NQO (2.5×10^{-4} mol dm^{-3} , pH 7.0) at 590 nm. Inset: Logarithmic plot of the absorbance vs. time.

From the slope of Figure 4, the self-quenching rate constant $k_{\text{sq}}(6.1 \times 10^8 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1})$ of ${}^T4\text{NQO}$ quenched by its ground state was deduced in neutral solution. The value of $1/\tau_T^0$ has been obtained from the intercept of Figure 4.

The kinetic parameters of ${}^T4\text{NQO}$ at other pH values have been obtained by the same data processing method as shown in Figure 5 and listed in Table 1. It is obvious from Table 1 that the k_{sq} was decreased with increase of the solution acidity. Because 4NQO is a molecule with weak basicity and it is easy to combine with a proton forming 4NQOH^+ cation in acid solutions, hence the collision probabilities between cations should be decreased and the self-quenching rate constants are notably reduced in strong acid solutions.

The intrinsic triplet lifetime of 4NQO in neutral solutions is evaluated as $31 \pm 1 \mu\text{s}$ from the decay at 590nm. There are a little changes for τ_T^0 in various pH and

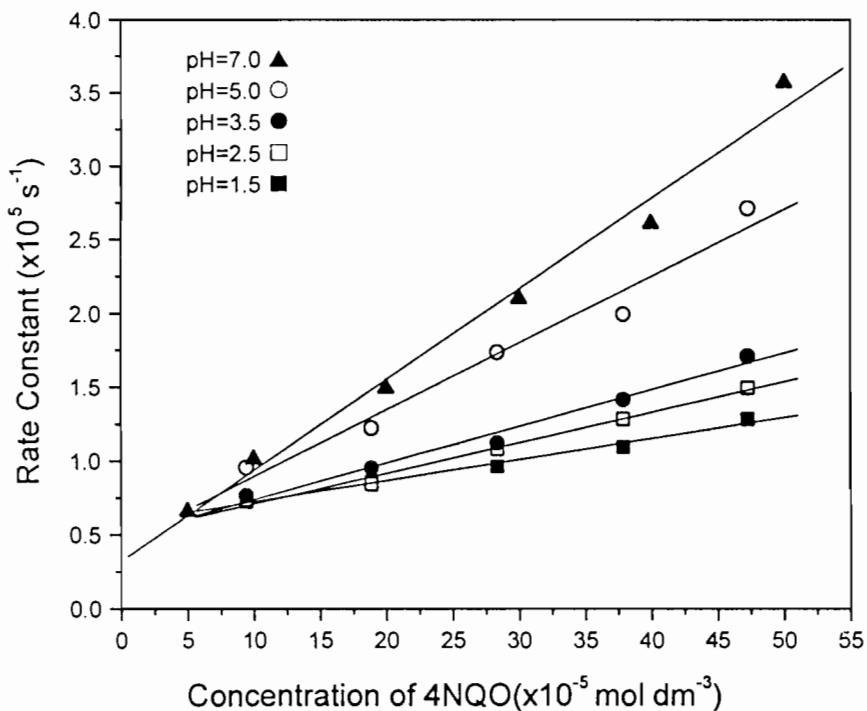


Figure 4. Plot of the decay rate constant of ^T4NQO vs. the concentration of 4NQO at different pH values.

the changing trend is similar to k_{sq} .

Photochemical Reactions of ^T4NQO with Amino Acids and Dipeptides

Neutral aqueous solution. The quinoline ring of 4NQO is electrophilic due to electron-withdrawing capacity of 4-nitro group and 1-oxygen. As compared with ground state, the triplet excited state of 4NQO is a stronger electrophile as electron density is decreased. Therefore, electron transfer would occur from amino acids to triplet 4NQO. According to Rehm-Weller equation [13], the free energy change (ΔG) for electron transfer between triplet 4NQO and amino acids can be calculated.

$$\Delta G \text{ (kJ}\cdot\text{mol}^{-1}\text{)} = 96.48 \times (E_{\text{ox}} - E_{\text{red}} - e^2/\epsilon d) - \Delta E_{0,0} \quad (3)$$

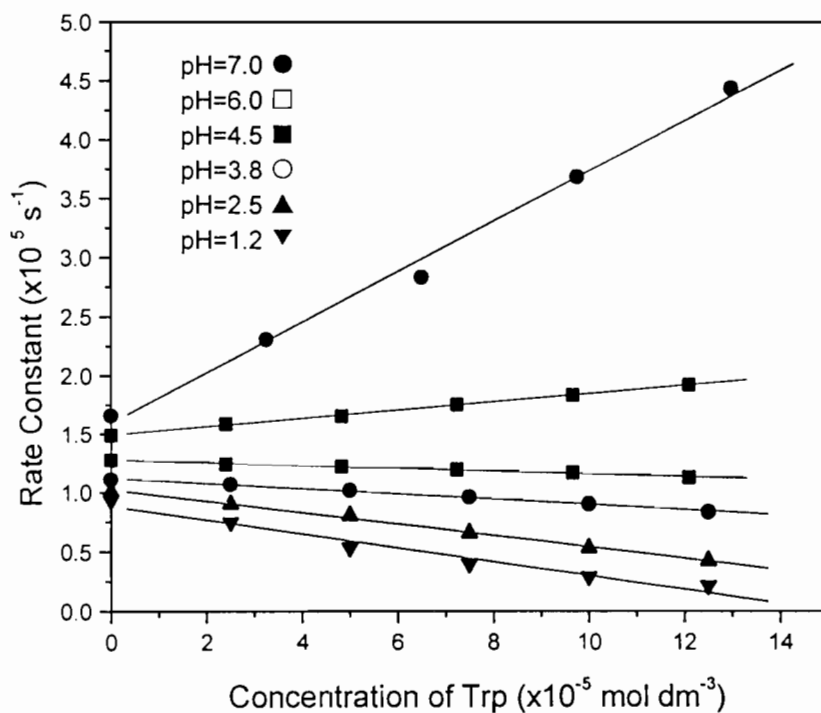


Figure 5. Plot of the decay rate constants of ${}^T4\text{NQO}$ vs. the concentration of Trp containing $2.5 \times 10^{-4} \text{ mol dm}^{-3}$ of 4NQO at different pH values.

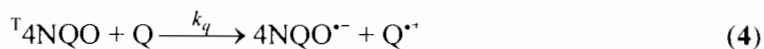
Table 1

Intrinsic triplet lifetime (τ_T^0) and self-quenching rate constant (k_{sq}) of ${}^T4\text{NQO}$ at different pH.

pH	$k_{sq}/\text{mol}^{-1}\text{dm}^3\text{s}^{-1}$	τ_T^0 (μs)
1.5	1.4×10^8	18
2.5	2.1×10^8	20
3.5	2.5×10^8	21
5.0	4.5×10^8	23
7.0	6.1×10^8	31

Where the coulombic term $e^2/\epsilon d$ can be neglected in aqueous solution. E_{ox} and E_{red} are the oxidation potential of the electron donor and the reduction potential

of the electron acceptor respectively. The measured E_{red} value of 4NQO is -0.37V (vs. SCE). $\Delta E_{0,0}$ is the lowest energy level of the triplet, 178 kJ mol^{-1} [6] for ${}^1\text{4NQO}$. The E_{ox} values of amino acids and dipeptides, as well as the ΔG values, are shown in Table 2. The ΔG values show that the electron could efficiently transfer from quencher Q (Trp, Tyr and dipeptides containing Trp or Tyr residue) to ${}^1\text{4NQO}$.



In the presence of a quencher (Q), the decay rate constant is

$$1/\tau_T = 1/\tau_T^0 + k_{\text{sq}}[4\text{NQO}] + k_q[\text{Q}] \quad (5)$$

Quenching rate constants were obtained from photolysis of 4NQO with amino acids and dipeptides, and listed in Table 2. The data in Table 2 show that k_q values of Trp, Tyr and dipeptides containing Trp or Tyr residue are in the range of $2.0 \times 10^9\text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$ to $2.6 \times 10^9\text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$, and $1.9 \sim 7.1 \times 10^7\text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$ for methionine and dipeptides without Trp and Tyr. These rate constants correlate well with the corresponding free energy changes.

Table 2

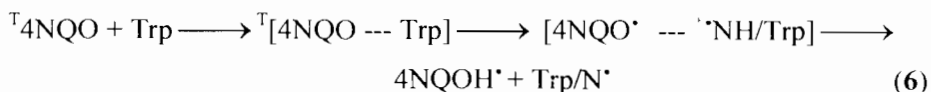
Quenching rate constants of ${}^1\text{4NQO}$ with amino acids and dipeptides in neutral solution, oxidation potentials and free energy changes^a of electron transfer reactions from quenchers to ${}^1\text{4NQO}$.

Quencher	$k_q/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	E_{ox}/V (NHE)	ΔG (kJ mol ⁻¹)
Trp-Tyr	2.6×10^9	0.91	-54.5
Tyr	2.4×10^9 3.1×10^9 ^a	0.93 0.93 ^b	-52.6
Tyr-Phe	2.3×10^9	0.95	-50.6
Trp	2.25×10^9 3.7×10^9 ^a	1.01 1.02 ^b	-44.8
Trp-Phe	2.2×10^9	1.06	-40.0
Trp-Gly	2.0×10^9	1.08	-38.1
Met-Gly	7.1×10^7	1.10	-36.2
Met-Phe	3.6×10^7	1.13	-33.3
Met	1.9×10^7 2.9×10^7 ^a	1.15	-31.3

^aFrom Ref. 7.

^bFrom Ref. 14.

Because the processes of protonation of ${}^1\text{4NQO}^{\bullet}$ and deprotonation of $\text{Trp}/\text{NH}^{+\bullet}$ (pKa 5.1 [14]) should proceed very fast in neutral solutions, the dissociation of the geminate radical ion pair $[\text{Trp}/\text{NH}^{+\bullet} \cdots \cdots {}^1\text{4NQO}]$ can occur simultaneously. The electron transfer process between ${}^1\text{4NQO}$ and Trp, Tyr as well as dipeptides containing Trp or/and Tyr could be expressed as:



The intramolecular electron transfer involving Tyr to $\text{Trp}/\text{N}^{\bullet}$ in model peptides $\text{Trp}(\text{X})_n\text{-Tyr}$ (where X is an amino acid residue) has been studied extensively by pulse radiolysis [15,16] and laser flash photolysis [17]. Hence, in photolysis process of ${}^1\text{4NQO}$ and dipeptides Trp-Tyr , an intramolecular electron transfer occurs, namely $\text{Trp}/\text{N}^{\bullet}\text{-Tyr} \longrightarrow \text{Trp-Tyr}/\text{O}^{\bullet}$, and finally forms $\text{Trp-Tyr}/\text{O}^{\bullet}$ radical. This implicates that similar transformation would exist in protein and $\text{Tyr}/\text{O}^{\bullet}$ residue is the final transient species in oxidation with ${}^1\text{4NQO}$.

The pH dependence. The decay rates of ${}^1\text{4NQO}$ quenched by Tryptophan were measured in pH lower than 7. The relation of decay rate to concentration of Trp is shown in Figure 5. It is found that the decay rate constant $1/\tau_T$ decreased with reduction of pH in the same concentration of Trp, and the relation of decay rate to concentration of Trp presented a straight line at each pH value.

The quenching rate constants k_q from the slope of straight lines were listed in Table 3. Here k_q values decrease with increase of solution acidity and become negative when $\text{pH} < 4.5$. We think two reasons can be used to explain these phenomena.

Firstly, the oxidation potential, E_{ox} , of Trp or Tyr raises with decrease of pH. For example, $E_{\text{ox}} = 1.05\text{V}$ (NHE) at $\text{pH} = 7$ and $E_{\text{ox}} = 1.13\text{V}$ (NHE) at $\text{pH} = 3$ for Trp, $E_{\text{ox}} = 0.76\text{V}$ (NHE) at $\text{pH} = 11$ and $E_{\text{ox}} = 0.94\text{V}$ (NHE) at $\text{pH} = 7$ for Tyr [14]. The free energy change for a photoinduced electron transfer reaction is given by Eq.3. ΔG value increases with E_{ox} raising. Thus the rate constant of electron transfer from Trp or Tyr to ${}^1\text{4NQO}$ decreases with E_{ox} raising.

Secondly, $\text{Trp}/\text{N}^{\bullet}$ radical has two absorption peaks, $\lambda_{\text{max}} = 330$ and 530 nm, in neutral solutions. When pH is less than 5, the peak at 530 nm is shifted to 580 nm and also the absorption at 580 nm increases with decreasing pH [18]. Therefore, in our experiment the 580 nm band of $\text{Trp}/\text{N}^{\bullet}$ also contributes to the absorption at 590 nm. As $\text{Trp}/\text{N}^{\bullet}$ radical is a long lifetime species, thus measured value of k_q becomes small in acidic solutions. The lower pH and the higher concentration of Trp make the

Table 3
Quenching rate constants of ^T4NQO by Trp and Tyr at different pH.

Quencher	pH	$k_q/\text{dm}^3\text{mol}^{-1}\text{s}^{-1}$
Trp	1.2	-5.8×10^8
	2.5	-4.8×10^8
	3.8	-2.2×10^8
	4.5	-1.2×10^8
	6.0	3.5×10^8
	7.0	22.5×10^8
Tyr	1.5	-6.6×10^8
	2.5	-5.6×10^8
	3.5	-4.0×10^8
	5.5	4.0×10^4
	7.0	23.9×10^8

larger influence of the 580 nm band.

The pH effects for photochemical reaction of 4NQO and Tyr are similar to the reaction of 4NQO and Trp. The quenching rate constants for Tyr are also listed in Table 3.

In case of $\text{pH} > 7$, the colour of the solution containing 4NQO and the amino acid became dark after laser pulses and no transient signal could be observed. This result shows that the amino acid was oxidized in alkaline solutions. Met is the most oxidizable one among Trp, Tyr and Met and was oxidized even at pH 8.5. Therefore, Met may be the initial reaction site when proteins reacted with ^T4NQO or ^S4NQO in weak alkaline solutions.

In summary, the self-quenching rate constants of ^T4NQO and the rate constants of electron transfer from amino acids to ^T4NQO decrease with decreasing pH in acidic solutions and amino acids are easily oxidized by 4NQO under laser irradiation in alkaline solutions.

Acknowledgements

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