

## Relationship between Singlet Oxygen Formation and Photolysis of Phloxine B in Aqueous Solutions

Young Soo Keum<sup>1</sup>, Jeong Han Kim<sup>2</sup>, and Qing Xiao Li<sup>1\*</sup>

<sup>1</sup>Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA

<sup>2</sup>School of Agricultural Biotechnology, Seoul National University, Seoul, Kwanak-Gu, Sinrimgu-Dong, San 56-1, Korea

Phloxine B (2,4,5,7-tetrabromo-4,5,6,7-tetrachlorofluorescein disodium salt), also referred as D&C red dye no. 28, is phototoxic to many insects such as Tephritidae fruit flies. Sunlight photolysis of phloxine B in aqueous solutions was a first order kinetic reaction at low concentrations. But it turned to be more complex reactions with the increase of phloxine B concentration. The half-lives of phloxine B (6-120  $\mu$ M) were 18-41 and 52-289 hours in oxygenated and deaerated distilled water, respectively. The photolysis rate constants increased as the phloxine B concentrations increased. The singlet oxygen formation positively correlated with the concentrations of phloxine B and humic acid in oxygenated distilled water. The formation of singlet oxygen did not stop even after the complete degradation of phloxine B, which suggested an involvement of photoproduct-mediated reactions. The results showed that singlet oxygen mediated photooxidation was a dominant reaction for phloxine B dissipation in an aqueous solution, and the self-sensitized and photoproduct-mediated reactions were also involved at the higher concentrations. Iodide and bromide ions significantly decreased phloxine B photolysis rate constants, which were in relation to the decrease of singlet oxygen formation.

**key words:** Xanthene dye, insecticide, singlet oxygen, phloxine B

### INTRODUCTION

Phloxine B (2',4',5',7'-tetrabromo-4,5,6,7-tetrachlorofluorescein disodium salt) (Fig. 1), also referred as D&C red dye no. 28, is a xanthene dye used in drugs and cosmetics [1]. Recent studies have shown that phloxine B, mixed in food bait, is highly toxic to Tephritidae fruit flies [2-6]. A possible toxic action is photooxidative damage of macromolecules in a biological membrane [7-9].

Methods were developed recently for the analysis of phloxine B and its analogues in soil and water [10,11]. Photobleaching

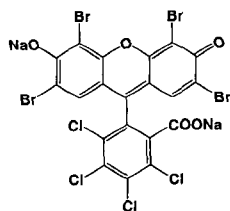


Figure 1. Structure of phloxine B

of related xanthene dye, such as rose bengal, eosine, erythrosine, and fluorescein was studied with oxygen [12-14]. It is known that xanthene dyes at low concentrations degrade rapidly through an oxygen-mediated photoreaction which requires dissolved oxygen, but a self-sensitized reaction predominates at high dye concentrations [12-14]. The photolysis rate constant of the above halogenated dyes was increased as increasing the degree of halogenation and the molecular weight of the halogens, and such molecular changes coincided well with the quantum yield of singlet oxygen production [15-17]. Photochemical behaviors of phloxine B in aqueous solutions under various light sources and in the presence of different halogen ions and concentrations were reported recently [18], which iodide ion greatly enhanced the photostability of phloxine B. The effect of halogen ions was rapidly diminished when the iodide was replaced by bromide, chloride, and fluoride. It is known that xanthene dyes generate singlet oxygen, however, the relation between singlet oxygen production and sunlight photolysis of phloxine B in aqueous solutions were not well studied.

In this study, various factors including oxygen, halogen ion, and humic acid were investigated for their effects on the photolysis of phloxine B. The relationship between singlet oxygen formation and photodegradation of phloxine B was studied.

\*To whom correspondence should be addressed.

E-mail : qingl@hawaii.edu

Received September 24, 2003; Accepted November 20, 2003

## MATERIALS AND METHODS

### Materials

Phloxine B was purchased from ICN Biochemicals (Cleveland, OH). High performance liquid chromatographic (HPLC) grade ammonium acetate ( $\text{NH}_4\text{OAc}$ ), methanol (MeOH), and water were purchased from Mallinckrodt Chemicals (St. Louis, MO). Sodium iodide, sodium bromide, sodium fluoride, 2,2,6,6-tetramethylpiperidine (TEMP), 2,2,6,6-tetramethyl-piperidone (TEMPO), and humic acid sodium salt were from Aldrich Chemical, Inc. (Milwaukee, WI).

### Photolysis of phloxine B

Sunlight photolysis experiments were performed on June 1-5, 2000 in Suwon, Korea (Latitude  $37^\circ$ ). Aqueous solutions of phloxine B (12 mL of 6, 12, 60, 120  $\mu\text{M}$ ) in a quartz tube (11 cm length  $\times$  1.2 cm i.d.) were oxygenated with oxygen for 20 min (oxygenated solution) or deaerated with helium purging for 10 min (deaerated solution). The sunlight intensity of  $1.49 \times 10^7 \text{ J/m}^2\cdot\text{day}$  (297 to 1100 nm) was measured by a LI-1800 spectroradiometer (Li-Cor, USA). An aliquot of samples (0.5 mL) was collected at different times after sunlight exposure, and analyzed immediately with HPLC. Phloxine B (60  $\mu\text{M}$ ) was dissolved in distilled water, humic acid solutions (5, 10, 50, and 100  $\text{mg L}^{-1}$  distilled water), or sodium iodide solutions (0.01, 0.1, and 1 M). Dark controls were covered with aluminium foil and placed under the same conditions as the treatments.

### Electron spin resonance spectroscopy

Singlet oxygen was trapped with TEMP (a final concentration of 15 mM) in the reaction solution [19, 20]. After the solution was exposed to a 400 W halogen lamp for 10 min, concentrations of TEMP, a radical adduct, were measured with electron spin resonance spectroscopy (ESR) (JEOL, JES-TE series, Japan). ESR was run at a microwave frequency of 9.4237 GHz, field 337.7 mT, mode width 0.02 mT, sweep time 1 min, microwave power 5 mV, amplification 400, and time constant 0.1 sec. Levels of TEMP indicating singlet oxygen concentrations were determined in distilled water and various solutions that contained phloxine B (0, 6-1200  $\mu\text{M}$ ), and humic acid (0, 10-500  $\text{mg L}^{-1}$ ), fluoride (0.05 M), bromide (0.05 M), or iodide ion (0.01-0.5 M).

### High performance liquid chromatography

Concentrations of phloxine B were determined with a Hewlett-Packard (HP) 1100 series HPLC equipped with a diode array detector and a Zorbax ODS column (250 mm length  $\times$  4.6 mm i.d., 5  $\mu\text{m}$  particle size). The mobile phase was a mixture of MeOH and 0.1 M  $\text{NH}_4\text{OAc}$  buffer with a linear gradient increase of MeOH from 60 to 100% within 30 min at a flow rate of 0.4  $\text{mL min}^{-1}$ . The detection wavelengths were 210 and 540 nm, and an injection volume was 100  $\mu\text{L}$ .

Table 1. Concentration dependent sunlight photolysis of phloxine B in oxygenated and deaerated distilled water<sup>a</sup>

Aeration	Phloxine B concentration, $\mu\text{M}$	$-k^b$ , $\text{hr}^{-1}$	$t_{1/2}$ , $\text{hr}^c$	$R^{2,d}$
Oxygenated	6	0.0169	41.0	0.9834
	12	0.0271	25.6	0.9587
	60	0.0358	19.4	0.9309
	120	0.0392	17.7	0.9301
Deaerated	6	0.0024	288.9	0.9872
	12	0.0069	100.5	0.9256
	60	0.0103	67.2	0.9982
	120	0.0131	52.9	0.9737

<sup>a</sup>Average values of two independent experiments.

<sup>b</sup> $k$  is photolysis rate constant.

<sup>c</sup> $t_{1/2}$  is photolysis half-life.

<sup>d</sup> $R^2$  is coefficient of determination.

## RESULTS AND DISCUSSION

### Concentration-dependent direct photolysis of phloxine B

Table 1 shows average half-lives ( $t_{1/2}$ ) and photolysis rate constants ( $-k$ ) of phloxine B at different concentrations in oxygenated and deaerated distilled water under sunlight. Photoreactions of phloxine B followed the first order kinetics at lower concentration, which confirmed the earlier observation [18]. However, the steady increase of photolysis rate constant suggested that a more complex reaction mechanism was involved in the photolysis of phloxine B at higher concentrations. Photolysis rate constants ( $-k$ ) approximately doubled as phloxine B concentrations increased from 6 to 12  $\mu\text{M}$  (Fig. 2A and Table 1), which agreed well with previous observations for other xanthene dyes such as erythrosine, eosine and uranine [12]. However, the rate constant increase was quite small (10-27%) as phloxine B concentrations increased from 60 to 120  $\mu\text{M}$ . It appeared that singlet oxygen generation coincided well with the photolysis rate constant changes (Fig. 2A and B). Such a relationship between the phloxine B concentration and

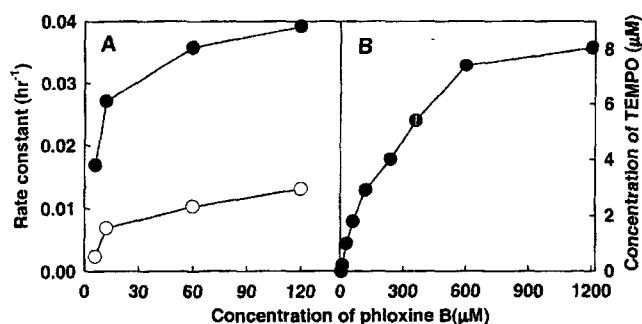


Figure 2. Relationship between concentrations and photolysis rate constants of phloxine B in oxygenated (●) and deaerated (○) distilled water (A) and singlet oxygen formation as monitored by TEMPO (B). The concentration of TEMPO was measured after 2 min of irradiation.

photolysis rate constant can be attributed to (a) oxygen depletion and subsequent switching to a different reaction mechanism (e.g., self-sensitization) at the high concentrations of the dye [12-14, 17, 21] and (b) quenching of singlet oxygen by the dye without chemical reactions [20]. In addition, bromide and chloride ions from phloxine B decomposition, particularly at its high concentrations, can retard singlet oxygen generation (results below), and thus decrease the photolysis constant. It is noted that no phloxine B degradation was observed in dark control samples during the experimental period.

#### Effect of oxygen concentration

Phloxine B was 3-7 times more stable in deaerated water than that in oxygenated water (Table 1, Fig. 2A), which was consistent with other xanthene dyes under similar conditions [12-14]. The results strongly indicated that singlet oxygen played a dominant role in phloxine B photolysis. Photodegradation under oxygen-free or limited oxygen conditions was reported with eosin and self-sensitization was suggested as a possible reaction mechanism [22]. An increased degradation rate constant of phloxine B at the higher concentrations in both oxygenated and deaerated distilled water suggests an involvement of a concentration-dependent self-sensitization (Table 1). However, the excited state dyes may be deactivated by ground state dyes at high concentrations, which can prohibit an infinite increase of the self-sensitizing reaction rate constant. The difference between the photolysis constants in oxygenated distilled water and those in deaerated distilled water became smaller (7.0, 3.9, 3.5, and 3.0 fold) as the phloxine B concentrations increased from 6 to 120  $\mu\text{M}$ , which suggests oxygen depletion effects and subsequent switching to self-sensitization at the higher concentrations of dye.

#### Effect of halogen ion

Photolysis of phloxine B was considerably retarded by iodide and bromide ions [18]. Debromination is a major initial reaction for phloxine B, by which bromide ion can be

accumulated, and affect the photodegradation. The ESR experiments showed that concentrations of TEMPO decreased concomitantly as increasing iodide molarity (Fig. 3), and the photolysis was inhibited by iodide ion in a concentration-dependent manner. Such an inhibition can be caused by (1) consumption of oxygen and singlet oxygen by iodide oxidation [23], (2) quenching of the dye from an excited state to a ground state [24, 25], and (3) heavy atom effect of iodide. Iodide is vulnerable to oxidation under environmental conditions, and thus it can act as a singlet oxygen scavenger. Singlet oxygen formation was also inhibited with bromide ion (Fig. 4). The results showed that the decrease of phloxine B photolysis rate constants by iodide and bromide ions was related to the decrease of singlet oxygen generation (Figs. 3 and 4).

#### Effect of reaction products

Two major initial degradation products were 2',4',5'-tribromo-4,5,6,7-tetrachlorofluorescein and 4',5'-dibromo-4,5,6,7-tetra-chlorofluorescein [18]. It is also known that di- and tri-bromofluorescein can retain 77 and 73%, respectively, of the singlet oxygen quantum yields as compared with 2,4,5,7-tetrabromofluorescein [16]. Therefore, the initial photoproducts of phloxine B can be singlet oxygen sensitizers. To investigate a possible involvement of reaction products in the singlet oxygen formation, concentrations of singlet oxygen and phloxine B were monitored simultaneously. The concentration of singlet oxygen reached maximum (1.41  $\mu\text{M}$ ) after 2 min of photoirradiation, and decreased rapidly (Fig. 5). Appreciable quantities of singlet oxygen (0.19  $\mu\text{M}$ ) were still detected after complete disappearance of phloxine B, and were possibly produced from the photosensitization of the reaction products because singlet oxygen was not detected in distilled water without phloxine B. Again, it should be noted that bromide and chloride ions from phloxine B degradation decreased singlet oxygen generation (Fig. 4) and slowed down the reaction [18]. Phloxine B photolysis can be further

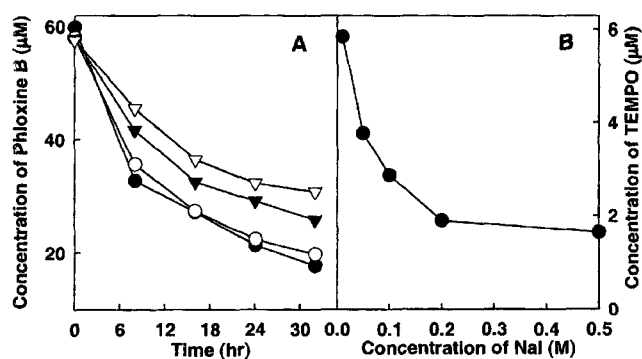


Figure 3. Inhibitory effect of iodide ( $\bullet$  0.00,  $\circ$  0.01,  $\blacktriangledown$  0.10, and  $\triangledown$  1.00 M) on sunlight photolysis of phloxine B (A) and on the singlet oxygen formation as monitored by TEMPO (B). The concentration of TEMPO was measured after 2 min of irradiation.

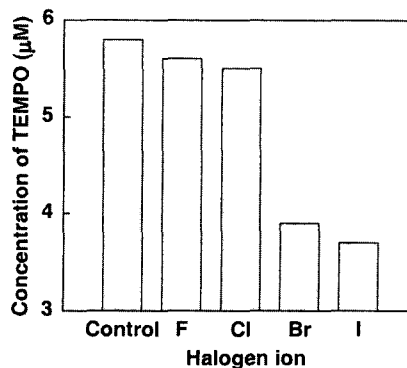


Figure 4. Effects of halogen ion species (F, Cl, Br<sup>-</sup> and I<sup>-</sup>, 0.05 M) on singlet oxygen formation in presence of 120  $\mu\text{M}$  phloxine B. The control was 120  $\mu\text{M}$  of phloxine B without halogen ion. The concentration of TEMPO was measured after 2 min of irradiation.

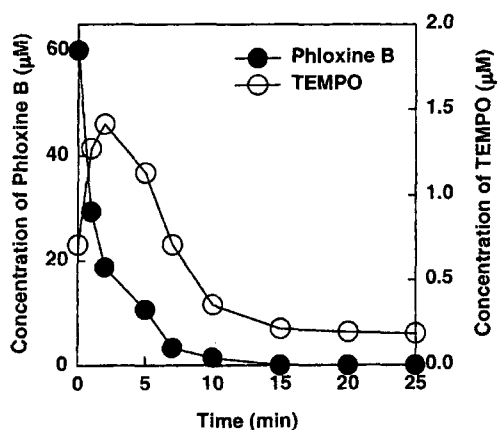


Figure 5. Photodegradation of phloxine B under halogen lamp (400 W) and formation of singlet oxygen as monitored with TEMPO.

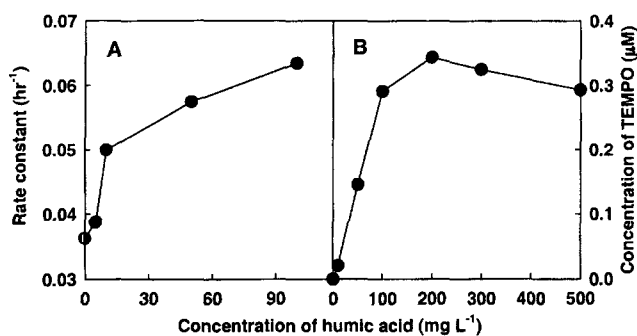


Figure 6. Relationship between humic acid concentrations and phloxine B photolysis constants (A) and singlet oxygen production (B).

complicated by self-sensitization at high concentrations.

#### Effect of humic acid

Humic acid exists ubiquitously in the environment. It contains various types of chromophores, and thus acts as strong photosensitizers to produce singlet oxygen and/or other reactive oxygens [26]. Humic acid accelerated photodegradation of phloxine B (Fig. 6A), and sensitized to produce an appreciable amount of singlet oxygen when phloxine B was not present (Fig. 6B). Singlet oxygen concentrations were correlated proportionally with humic acid concentrations (0–100 mg L<sup>-1</sup>), which matched well with the photolysis constant changes of phloxine B as a function of humic acid concentrations. It is known that a light-screening effect of humic acid may occur at high concentrations (> 100 mg L<sup>-1</sup>) [27].

## CONCLUSION

☞ Sunlight photolysis of phloxine B in aqueous solutions followed the first order kinetics at low concentrations of phloxine B. The half-lives of phloxine B (6–120 µM) were 18–41 and 53–289 hours in oxygenated and deaerated distilled water, respectively. The reaction constants increased 2.3-fold

(from  $16.9 \times 10^{-3}$  to  $39.2 \times 10^{-3}$  hr<sup>-1</sup>) when phloxine B concentrations in oxygenated distilled water increased from 6 to 120 µM. The reaction constants increased 5.5-fold (from  $2.4 \times 10^{-3}$  to  $13.1 \times 10^{-3}$  hr<sup>-1</sup>) when phloxine B concentrations in deaerated distilled water increased from 6 to 120 µM. The phloxine B degradation constants and profiles coincided well with those of singlet oxygen formation. Continuous formation of singlet oxygen after the complete degradation of phloxine B suggested that photosensitization by reaction products exist. Iodide and bromide ion inhibited singlet oxygen formation and phloxine B photolysis. The results showed that phloxine B at low levels in oxygenated distilled water was decomposed mainly by singlet oxygen produced through photosensitization by phloxine B and photoproducts. Phloxine B was decomposed rapidly in humic acid solution, and the photolysis constants correlated well with singlet oxygen formation as humic acid concentrations increased.

## Acknowledgements

This work was supported in part by USDA-ARS award number 58-5320-8-147.

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