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RESONANCE ENERGY TRANSFER FOR THE DONOR-ACCEPTOR PAIR CURCUMINE-METHYLENE BLUE AND SINGLET OXYGEN SENSITIZATION

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ABSTRACT. Resonance energy transfer takes place when a food dye, curcumine (*Curcuma longa*), is excited in the presence of a thiazine dye, methylene blue. In solution the energy transfer between curcumine and methylene blue does not obey Stern-Volmer kinetics, but using limiting slopes, a rate constant (k_ω) of 7.11×10^{11} L mol^{-1} s^{-1} and a critical distance (R_0) of 56.1 Å are obtained. In cellulose acetate polymer films, the rate constant of energy transfer is found to be 4.5×10^{10} L mol^{-1} s^{-1} with $R_0 = 39 \pm 2$ Å. The consequence of energy transfer when this donor-acceptor pair is excited in the presence of oxygen is singlet oxygen ($^1O_2$) production. This is observed from the enhancement of the quantum yield of photodegradation of the singlet oxygen acceptor, 2,5-diphenylfuran and by quenching of singlet oxygen by 1,4-diazabicyclo[2.2.2]octane.

INTRODUCTION

Resonance energy transfer refers to the donation of excitation energy from an excited donor chromophore (D^*) to the acceptor (X), resulting in the promotion of the acceptor to an excited electronic state [1] .

$$D^* + X \rightarrow D + X^* \qquad (1)$$

This process, together with the radiative mechanism (trivial), plays an important role in many photochemical and photobiological processes [1-9]. The Förster equation (2) adequately describes this process which has been useful in the understanding of the nature of reactive excited states, their energies, efficiency, and in attempts to simulate the light harvesting processes in photosynthesis [5, 9-12].

$$k_{D^*\text{-}X} = \frac{8.8\times10^{-25}K^2\Phi_D}{n^4\tau_D R^6}\int_0^\infty \frac{F_D(\bar{\nu})\varepsilon_X(\bar{\nu})d(\bar{\nu})}{\bar{\nu}^4} \qquad (2)$$

In equation 2, $\bar{\nu}$ is the wave number (in cm^{-1}), F_D ($\bar{\nu}$) is the spectral distribution of the donor emission (in quanta, expressed as a function of wave number and normalized to unity), ε_X($\bar{\nu}$) is the molar extinction coefficient for the acceptor absorption, n is the refractive index of the solvent, K^2 is an orientation factor which is equal to 2/3 for a random distribution of molecules, Φ_D is the quantum yield of the donor emission, τ_D is the donor emission lifetime (s) and R is the distance (Å) between the donor and acceptor molecules. $k_{D^*\text{-}X}$, is the rate constant of energy transfer and is related to R_0 by equation 3.

$$k_{D^*-X} = \frac{1}{\tau_D}\left(\frac{R_0}{R}\right)^6 \quad (3)$$

A number of approaches may be used to study energy transfer [1, 13-18]. One approach, which was also used in this work, involves measuring the fluorescence of the donor in the presence of varying amounts of the quencher. Quantum yields of donor fluorescence are then calculated using equation 4 [19] and energy transfer analyzed as will be described below.

$$\Phi_u = \frac{\Phi_s . A_u . n_u^2 . I_0^s}{A_s . n_s^2 . I_0^u} \quad (4)$$

The terms Φ, A, n and I in equation 4 refer to the quantum yield, integrated area under the peak, refractive index and intensity, respectively. Subscripts u and s refer to the unknown and the standard.

Once quantum yields are obtained, evaluation of energy transfer is done depending on whether energy transfer is occurring in solution or in the solid. For samples in solution, the easiest approach involves the use of Stern-Volmer kinetics [5, 8, 9, 17, 20-22] which may be described by the photochemical equations 5 - 8.

		Rate	
$D + h\nu \rightarrow D^*$		fI	(5)
$D^* \rightarrow D + h\nu$		$k_1[D^*]$	(6)
$D^* \rightarrow D$		$k_2[D^*]$	(7)
$D^* + X \rightarrow D + X^*$		$k_3[D^*][X]$	(8)

where, I is the intensity of light, f is the efficiency of light absorption and k_2 represents the sum of the rate constants for decay of D^* by all other processes except fluorescence (rate constant k_1) and quenching by quencher X (rate constant k_3 is the same as k_{D^*-X} in equations 2 and 3). Using steady-state approximations for $[D^*]$ and $[X^*]$ the Stern-Volmer relation is given by

$$\frac{\Phi_0}{\Phi_D} = 1 + \tau_D^0 k_3 [X] \quad (9)$$

where, τ_D^0 is the lifetime (measured or calculated from the 0-0 absorption peak) of D^* in the absence of quencher. A plot of Φ_0/Φ_X *versus* [X] should give a straight line with a slope of $k_3\tau_D^0$. However, there are a number of cases reported where the simple Stern-Volmer kinetics does not adequately model the energy transfer in solution, thus requiring modifications [22, 23]. The important thing about equation 9 is that the concentration of the quencher at half value concentration ($[X]_{1/2}$, also referred to as the critical concentration, C_X^0) is easy to determine; and this is related to the critical distance R_0 between the donor and acceptor by equation 10

$$R_0 = 3\sqrt{\frac{3000}{4\pi N [X]_{1/2}}} = 7.35[X]_{1/2}^{-1/3} \quad (10)$$

where N is the Avogadro's number.

In the solid-adsorbed state, evaluation of energy transfer parameters may be done on the basis of Förster's derived luminescence equation for the solid or solutions of moderate viscosity [1, 10, 24].

$$\frac{\Phi_X}{\Phi_0} = 1 - P_{D^*-X} \tag{11}$$

where

$$P_{D^*-X} = \sqrt{\pi} x e^{x^2}[1 - erf(x)] \tag{12}$$

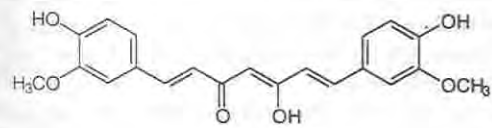
and

$$x = \sqrt{\frac{\pi}{2}\frac{C}{C_0}} \tag{13}$$

C is the reduced acceptor concentration, erf(x) is the error function for x, and x is a dimensionless form of the acceptor concentration.

In the study of energy transfer, an important area which has not been given attention is the area of singlet oxygen sensitization. This may be attributed to the fact that usually experimental designs for energy transfer studies (especially in solution) involve working with oxygen free matrices since oxygen is known to quench some excited states [2, 5, 24].

Curcumine is a natural dye which is usually extracted from the rhizomes of various *Curcuma* species (Zingiberaceae). This dye is widely used as a colouring agent in food, drugs and cosmetics. It has various pharmacological effects [25, 26] and its practical and optical utility as a pharmaceutical additive have also been reported [27, 28]. This dye, however, is a poor sensitizer of singlet oxygen [8]. This paper reports on how resonance energy transfer from curcumine to Methylene Blue (Figure 1), measured from quenching of donor fluorescence, may be used for improved singlet oxygen sensitization. It is believed that this mode of light trapping (using curcumine as antennae) and consequent harvesting (by good sensitizers such as methylene blue) could have important applications in singlet oxygen mediated processes such as photodynamic therapy [29-40], oxygen scavenging [8, 41-46] and in polymer photo-degradation and stabilization [47-50].



Curcumine (CUR)

Methylene Blue (MB)

Figure 1. Molecular structures of curcumine and Methylene Blue.

EXPERIMENTAL

Reagents. Chloroform, absolute ethanol and acetone (Analar, BDH) were used as received. Perchloric acid (high quality spectro grade), sodium nitrite (Analar, BDH), 2,5-diphenylfuran (DPF) (Analar, BDH), 9,10-diphenylanthracene (DPA) (Scintillation grade from Koch-Light laboratory), 1,4-diazabicyclo[2.2.2]octane (DABCO) (Analar, BDH) and cellulose acetate (Ajax Chemicals) were also used as received. Sodium fluorescein (NaFl) (Gurr microscopy, BDH) and Rhodamine B (RhB) (spot test reagent, C. I. 45170) were recrystallised from ethanol and dried in a vacuum desiccator. Curcumine (CUR) was recrystallised from water. Methylene Blue (MB) (Analar, BDH) was purified by repeated recrystallisation from ethanol and dried in the oven at 105°C and was therefore assumed to be in monomer form [51]. The Reinecke's salt [$K(Cr(NH_3)_2(NCS)_4]$ and iron(III) nitrate, which were used in actinometry, were recrystallized three times from water [52]. For all the dyes purity was checked by absorption spectrophotometry and thin layer chromatography.

Stock solutions. Stock solutions of CUR (5×10^{-3} M) and MB (10^{-2} M) were prepared in ethanol and that of DPF (10^{-2} M) was made in chloroform. Samples (solutions and films) consisted of 10^{-3} M CUR and varying amounts of MB.

Polymer films. Cellulose acetate (CA) films were cast under dim light in a dark room on a K-coater RK Print Coat Unit No. 41889 equipped with a micrometer (L. S. Starret Co. Ltd., Great Britain, No. 263) and an electrically driven casting bar, fitted with a clutch (Citenco F. H. P. Motors, Type KQT/35). The films were cast as follows: A 10% w/v CA solution was prepared by placing 2 g of CA in a 25 mL beaker and adding 20 mL of acetone. Appropriate amounts CUR and MB were added and the mixture stirred gently with a glass rod to make a slurry. The slurry was left to stand until release of occluded air bubbles. The polyester film (Mylar) was then spread onto the caster plate and the distance between the casting bar and the surface of the plate adjusted with a feeler gauge (Vitrex, England, 80.05-0.50 mm, No. 1911). The polymer solution was poured onto the caster plate and the motor engaged to cast the film. The dried film was stored in foil laminate bags. The thickness of the films was measured with a micrometer (Erichsen Model 497, D-5870 from Erichsen GMBH & Co. Hemer-Sundwig, W. Germany, precision; ± 0.5 μm).

Spectrofluorometry. Fluorescence and excitation spectra were measured on a Perkin Elmer MPF 44B fluorescence spectrofluorometer, equipped with an Osram 150 W HBO1 high pressure xenon-arc lamp, at room temperature (22°C). The fluorescence detector was a Hamamatsu R928 photomultiplier. A Perkin Elmer MPA amplifier and a Houston Instruments Omniscribe recorder were used. Solution samples were placed in 0.2 cm path length cuvettes which were kept in a firmly mounted PVC cuvette holder to enable spectra to be recorded at the same angle each time. Film samples were mounted on thick manilla paper frames and placed in a film holder that ensured illumination at the same angle. The excitation and emission slit widths were 5 and 1.0 nm, respectively. Fluorescence was observed from the light exposed side, with the cuvette or film at an angle of 30° to the incident light. The wavelength of exciting light used in the determination of fluorescence spectra was 410 nm. The emission wavelength for CUR excitation was 500 nm. To remove stray light, sharp cut-off filters (CS2-73, corning works), filtering at 430 nm were used. Emission and excitation spectra were corrected for spectral response of the photomultiplier and spectral distribution of lamp energy using a tungsten halogen lamp (Tungsram 50500T5, 12 V, 60 W, operating at a colour temperature of 3100 ± 100 K) powered by a regulated DC power supply (Power master, Model

T4000, with AC input, 240 V, 50 Hz and DC output 13.8 V, 4 A). This was done by recording the apparent spectra (instrumental output) and correcting the signals at each wavelength by dividing them by the corresponding values of the relative spectral quantum distribution determined by standard procedures [13, 53-56]. The emission spectrum of CUR was also corrected for reabsorption using suitable reabsorption factors, $R_n(\lambda)$, which were derived using equation 14 [13, 57, 58].

$$R_n(\lambda) = \frac{\mu(\lambda_{exc})\sin\beta\{1-\exp[-\delta c\frac{\mu(\lambda_{exc})\sin\beta + \mu(\lambda_{em}\sin\alpha)}{\sin\beta}]\}}{\mu(\lambda_{exc})\sin\beta + \mu(\lambda_{em})\sin\alpha(\lambda - \exp[-\delta c\mu(\lambda_{exc})]} \quad (14)$$

In equation 14, $\mu(\lambda)$ is the molar absorption coefficient at wavelength λ ($\mu = 2.303\ \varepsilon$ and ε is the molar extinction coefficient), δ is the penetration depth of the excitation beam in the sample (cm), and c is the concentration (mol L^{-1}). The angles α and β are related to the angles α^* and β^*, that the excitation beam and the emission beam, respectively, make with the face of the cell or film by the Snell refraction law (equation 15),

$$\frac{\cos\alpha^*}{\cos\alpha} = \frac{\cos\beta^*}{\cos\beta} = n \quad (15)$$

where n is the refractive index of the solvent.

Fluorescence quantum yields of CUR in solution were based on the value 0.79 for 10^{-3} M NaFl in 0.1 M NaOH [59, 60] and 0.98 for 9,10-diphenylanthracene [60]. Because of lack of a suitable time resolved spectrofluorometer, the lifetimes were estimated from the absorption spectrum of CUR (0-0 absorption peak at 430 nm) using the method developed by Förster [5, 9, 10]. The lifetimes determined in this way are known to compare well with measured values [5, 11]. For the film samples, fluorescence quantum yields were based on RhB [$\Phi = 0.93$] also dissolved in CA [60].

Absorption spectra and light exposure. Absorption spectra were recorded on a Perkin Elmer Lambda 2 UV/VIS spectrophotometer. Samples were irradiated with Osram HBO 200 W super pressure mercury lamps. These lamps were housed in a Bausch and Lomb SP 200 lamp holder, which was attached to a Bausch and Lomb high intensity monochromator (Cat No. 33-86-76). A spatial glass filter was used to cut off stray light at the exit slit of the lamp holder. The lamp was powered by a separately housed transformer (115 V, 60 Hz) and was always allowed five minutes to reach its operating equilibrium temperature before use. Light from the source was led to the monochromator, then through an optical lens, a filter solution (saturated sodium nitrite) and to the cuvette holder. All these components were mounted on a triangular optical bench. During irradiation solution samples were shaken every 2 min, and each sample was irradiated in such a way that less than 10% conversion was allowed in order to avoid the possibility of actinically active photoproducts interfering with the absorption characteristics of the samples [61].

RESULTS AND DISCUSSION

The emission and absorption spectra of CUR and MB are shown in Figure 2. The emission spectrum of CUR (normalized at 520 nm) shows good overlap with the absorption spectrum of MB (the overlap integral is 3.4×10^{-13} (equation 2)).

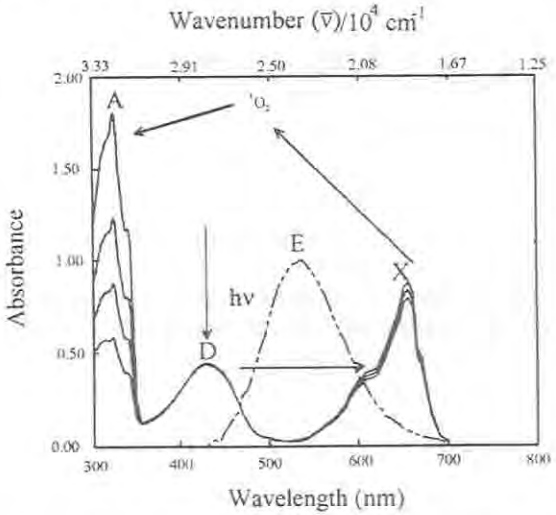


Figure 2. The absorption spectra of CUR (D), MB (X) and the emission spectrum of CUR (E).

CUR was found to fluoresce with a quantum yield of 0.17 ± 0.06 in ethanol. In acetonitrile, a quantum yield of 0.11 ± 0.05 was obtained. This value compares well with a reported fluorescence quantum yield value of 0.104 for CUR in acetonitrile [62]. The difference between the values in ethanol and acetonitrile is because the different solvent media are known to affect electronically excited states and associated processes such as internal conversion, fluorescence, intersystem crossing efficiency and phosphorescence [5, 11, 21].

Figure 3 is the Stern-Volmer plot for quenching of 10^{-3} M CUR fluorescence in solution. The plot is curved instead of being linear as expected from the Stern-Volmer kinetics. Energy transfer was therefore determined from the limiting slope [14]. Accordingly, the half value concentration ($[X]_{1/2}$) was found to be 2.25×10^{-3} M, and the limiting slope was 4.05×10^{2}. When these values and the lifetime of CUR (3.37×10^{-9} s from the 0-0 absorption peak) are put in equations 9 and 10, it is found that $R_o = 56.1$ Å and $k_{et} = 7.11 \times 10^{-11}$ L mol^{-1} s^{-1}. From the overlap integral for CUR and MB (3.4×10^{-13}), R_o is calculated to be 43.7 Å, while k_{et} is 10.7×10^{10} L mol^{-1} s^{-1} which are of the same order of magnitude as the experimental values. The experimental value of k_{et} is seven times more than the calculated value because diffusion of molecules as a result of low viscosity of the solvent enhances the rate of energy transfer. This also explains the non linearity of the Stern-Volmer equation at higher quencher concentrations. The energy transfer parameters obtained in solution are consistent with a process which occurs through a resonance mechanism, and are comparable with the values obtained in plastic films (see Table 1), where diffusion is avoided because of rigidization of the donor and acceptor molecules. Detailed discussions of this behaviour has been adequately given in the literature [2, 14].

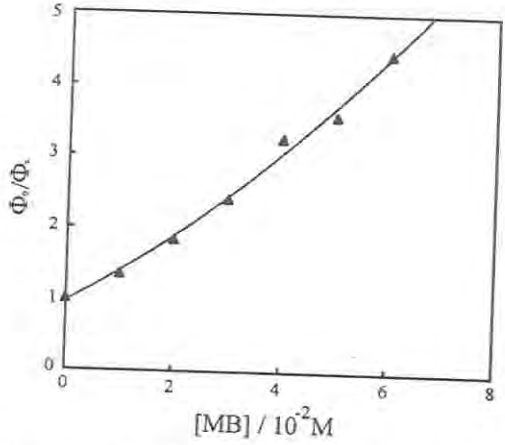


Figure 3. Stern-Volmer plot for quenching of quantum yields of 10^{-3} M CUR fluorescence by MB in ethanol.

The fluorescence quantum yield of CUR in the films was found to be 0.25 ± 0.05. The high fluorescence quantum yield in the film compared to the solution is in accordance with the lowering of the rate of internal conversion when molecules are rigidized [63-65]. This enhances fluorescence and is the basis for determining metals in solution by chelation. The results for energy transfer between CUR and MB in cellulose acetate films are shown in Table 1. The R_o values were derived from quantum yields by evaluating the error function using tabulated values [66] in conjunction with a fixed point iteration method.

Accordingly, the concentration of the quencher is varied widely by a factor of 6, and yet the derived value of R_o is constant at 39 ± 2 Å, within a probable error of 5%. This variation is a reasonable estimate of experimental statistical error. The value also compares well with the value of 43.7 Å calculated from the overlap integral. In solution, however, the R_o value was 56.1 Å, which is higher than the value in the films. When the R_o for the film is substituted in equation 2, the rate of energy transfer is found to be 4.5×10^{-10} L mol^{-1} s^{-1}, which is smaller than the value obtained in solution again due to less effects of diffusion in the films. In both media, however, the R_o values are larger than the normal collisional distances (10-15 Å). This, coupled with the sensitized fluorescence of MB as CUR fluorescence is quenched, confirms that energy transfer takes place by a resonance mechanism [1, 9, 21, 67].

When MB was added to CUR solution, no changes in the shapes of the spectra were observed. This implies that neither excimer nor ground state formation take place when MB is added to CUR [11].

MB is a well known sensitizer of singlet oxygen. Therefore, it is expected that energy transfer from CUR to MB should result in singlet oxygen production. The production of singlet oxygen was confirmed in this work from the photodegradation of a suitable singlet oxygen acceptor, DPF (reactive singlet oxygen acceptor, $k_r = 9 \times 10^7$ L mol^{-1} s^{-1}), and by quenching of singlet oxygen with DABCO [49, 50]. This was done by adding DPF (5×10^{-5} M in solution and 3×10^{-2} M in 20 μm cellulose acetate films) to mixture of CUR and MB and irradiating at the maximum absorption peak of CUR (430 nm) as illustrated by the arrows in Figure 2.

Table 1. Quenching of CUR fluorescence by MB in 20 μm cellulose acetate films.

[CUR]/M	[MB]/M	Φ_x/Φ_o	P_{D^*-A}	x	R_o(Å)
10^{-3}	0	1.00	0.00		
10^{-3}	$1x10^{-3}$	0.74	0.26	0.18	41
10^{-3}	$2x10^{-3}$	0.62	0.38	0.29	38
10^{-3}	$3x10^{-3}$	0.48	0.52	0.46	39
10^{-3}	$4x10^{-3}$	0.39	0.61	0.62	39
10^{-3}	$5x10^{-3}$	0.36	0.64	1.67	37
10^{-3}	$6x10^{-3}$	0.32	0.68	0.76	37

The mean experimental value of $R_o = 39 \pm 2$ Å.

Table 2. Quantum yield for CUR-MB sensitized photooxidation of DPF (Φ_{DPF}) in solution and in cellulose acetate films.

[MB]/10^{-4} M	Φ_{DPF}*†	[MB]/10^{-3} M	Φ_{DPF}*‡
0.00	0.036	0.00	0.25
0.40	0.038	0.20	0.26
1.00	0.040	0.40	0.26
2.00	0.042	0.60	0.28
4.00	0.056	0.80	0.30
6.00	0.061	1.00	0.32
8.00	0.065	2.00	0.33
10.00	0.070	3.00	0.35
		4.00	0.36
		5.00	0.33

* Relative error = 8.0%, † photooxidation of $3x10^{-4}$ M DPF in solution, and, ‡ photooxidation of $2x10^{-2}$ M DPF in 20 μm cellulose acetate films.

Under the concentration used in this work, and at the wavelength of irradiation, light was absorbed predominantly by CUR (ε = 49000 L mol^{-1} cm^{-1} at 430 nm), because MB has a low extinction coefficient at this wavelength (ε = 1200 L mol^{-1} cm^{-1} at 430 nm). This energy is then passed to MB which sensitizes singlet oxygen more efficiently than CUR. The efficiency of singlet oxygen sensitization increases with increase in quenching of energy by MB. This therefore, results in the enhancement of the quantum yield of singlet oxygen sensitization as observed from the increase in the quantum yield of DPF photodegradation shown in Table 2.

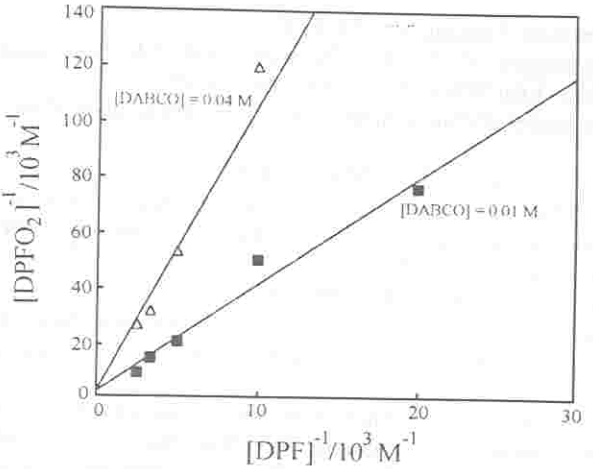


Figure 4. Quenching of the CUR-MB energy transfer sensitized photooxidation of DPF by DABCO in solution.

Quantum yields in the films are generally higher than the yields in solution, because rigidization of molecules by dissolving in a polymer enhances intersystem crossing efficiency (higher triplet quantum yields) and thus, greater singlet oxygen production [64, 65]. The involvement of singlet oxygen in the photodegradation of DPF was further confirmed by irradiating samples at the CUR peak in the presence of varying amounts of DABCO. The results (Figure 4) show that with variation of DABCO concentrations, plots of $[DPFO_2]^{-1}$ *versus* $[DPF]^{-1}$ give straight lines, with same intercepts but with different slopes. This therefore confirms that the intermediate species in the photooxidation of DABCO by this method of singlet oxygen sensitization is the same [49]. The quenching constant (k_q) of DABCO was determined as $4.3 \times 10^6\ M^{-1}s^{-1}$, in agreement with the literature value of $6 \times 10^6\ M^{-1}s^{-1}$ [68].

Thus, excitation of CUR in the presence of MB results in energy transfer by resonance, and this, when it occurs in the presence of oxygen, leads to sensitization of singlet oxygen. This phenomenon is believed to be similar to the light harvesting process that occurs in photosynthesis [3, 8, 9]. The increase in the quantum yields of singlet oxygen sensitization with increase in quenching of CUR fluorescence as observed in this work could in principle be useful for enhancing processes that take place via singlet oxygen sensitization.

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