The Laser Photolysis of Methylene Blue in the Presence of *p*-Chlorophenylglycine

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(Received September 20, 1971)

The photoreduction of methylene blue by a ruby laser light has been studied. The kinetic treatment and the measurement of the apparent activation energy show that, under the high-intensity irradiation, the excited triplet state of methylene blue plays a main role in the photoreduction by *p*-chloro phenylglycine. The apparent activation energy of the reduction of methylene blue is reduced from 7.4 to 2.8 kcal as the laser light energy increases from 23 to 530 mJ.

The photoreduction of methylene blue (MB) has been extensively studied by continuous irradiation[1-5] and by flash photolysis.[6-9] From these studies, it has become apparent that the photoreduction of MB by a reducing agent such as *N*-phenylglycines proceeds through the interaction between the lowest triplet state of MB and the reducing agent.

In a previous paper,[10] the saturation of the light absorption was observed using a giant-pulse ruby laser. Under such circumstances, the photoreduction of MB in the presence of dimethyl-phenylglycine (DMPG) has been proposed to take place through the excited triplet state of MB, which itself results from the subsequent photon absorption by the lowest triplet state.

The present paper is concerned with the reinvestigation of the excited triplet-state mechanism of MB using *p*-chloro-phenylglycine (*p*-CPG) as the reducing agent, for it is a mild reductant; the quantum yield of the photoreduction[2] of MB by *p*-CPG of an infinite concentration is 0.185, while that by DMPG is 0.388 at pH 7.0. Furthermore, new experimental proof of the excited-triplet-state mechanism was obtained from the apparent activation energies of the reduction of MB corresponding to two kinds of triplet states.

Experimental

The ruby laser head used in this work was the NEC model O-N583, which accommodates a ruby rod 8 mm in diameter and 150 mm long. The Q-switch accomplished by using an ethanol solution of cryptocyanine. The laser pulse 6943 Å was *ca.* 20 nsec in duration and had an energy of *ca.* 530 mJ, which corresponds to 1.85×10^{18} photons. The cross-sectional area of the laser beam was *ca.* 0.25 cm². Several neutral density filters, whose transmittance percentages were calibrated for the laser light, were used to reduce the light intensity. The quartz reaction cell used was cylindrical, 13 mm in diameter and 18 mm in path length.

Commercially available MB was obtained from the Koso Chem. Co. and was recrystallized from ethanol and water. The *p*-CPG was kindly offered by Professor Shiro Matsumoto. Dye solutions were prepared in borate buffer solutions in order to keep the pH at 8.0, at which the quantum yield of the photoreduction was at its maximum.[2,3] Degassing was carried out by freeze-pump-thaw cycles. The concentrations of MB were determined from the reported extinction coefficient[11] at the absorption peak ($\varepsilon=9.5\times10^4$ at 665 mμ). The energy of the laser pulse was measured by means of a TRG-100 thermopile. The temperature of the reaction cell was regulated by means of an electric furnace.

Results and Discussion

The transmitted light intensity (I_T) was measured as a function of the incident light intensity (I_0) for the MB solution without the reducing agent; the results are shown in Table 1. No reaction was observed under the laser-pulse irradiation. Using the value of 1.3×10^4 as the extinction coefficient of MB[11] at 6943 Å, the I_T/I_0 ratio was estimated to be 0.70 for the 6.6×10^{-6} M solution. However, the experimental results show a much higher transparency than the value estimated above. This indicates that the saturation of the laser light absorption is realized in the MB solution without the reducing agent. Since the radiative lifetime of the MB singlet-excited state is estimated to be 9.8 nsec from the spectral data[11], and since the fluorescence quantum yield is quite low,[12] the actual lifetime of this state is at least an order of magnitude smaller

TABLE 1. THE TRANSMITTANCE OF MB SOLUTION AND THE NUMBER OF ABSORBED PHOTONS AS A FUNCTION OF THE DOSE LIGHT INTENSITY

I_0 (mJ)	I_T (mJ)	I_T/I_0	$N_{abs.}$ (photons/molecule)
530	477	0.88	87
440	392	0.86	79
244	215	0.83	48
66	59	0.78	16
23	19	0.75	7

$[MB]=6.6\times10^{-6}$M

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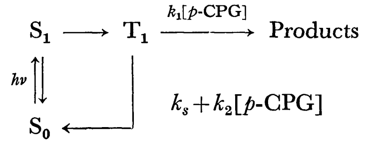
[1] G. Oster and N. Wotherspoon, *J. Amer. Chem. Soc.*, **79**, 4836 (1957).
[2] S. Matsumoto, This Bulletin, **35**, 1860 (1962).
[3] S. Matsumoto, *ibid.*, **35**, 1866 (1962).
[4] M. Koizumi, H. Obata, and S. Hayashi, *ibid.*, **37**, 108 (1964).
[5] N. Kosui, K. Uchida, and M. Koizumi, *ibid.*, **38**, 1958 (1965).
[6] C. A. Parker, *J. Phys. Chem.*, **63**, 26 (1959).
[7] S. Kato, M. Morita, and M. Koizumi, This Bulletin, **37**, 117 (1964).
[8] S. Matsumoto, *ibid.*, **37**, 491 (1964).
[9] S. Matsumoto, *ibid.*, **37**, 499 (1964).
[10] I. Tanaka, Y. Mori, Y. Minagawa, and E. Okutsu, *J. Phys. Chem.*, **72**, 2684 (1968).
[11] K. Bergmann and C. T. O'koshi, *J. Amer. Chem. Soc.*, **67**, 2169 (1963).
[12] N. Wotherspoon and G. Oster, *ibid.*, **79**, 3992 (1957).

than the radiative lifetime. Actually, the fluorescence lifetime of MB has been directly measured to be 0.4 nsec using a mode-locked He-Ne laser.[13] This short lifetime indicates that the intersystem crossing rate is quite fast. Thus, in an early stage of the laser-pulse duration, almost all the MB molecules are probably stored in the lowest triplet state. As the absorption coefficient of the lowest triplet state is lower than that of the ground state[14] at 6943 Å, the saturation of the laser-light absorption results from the absence of the ground-state molecules in the pulse duration.

The last column in Table 1 shows the number of photons absorbed (N_{abs}) by each MB molecule. The number of photons absorbed increases with the light intensity; at 530 mJ, one MB molecule absorbs nearly 90 photons. This may be interpreted by the following repetitive process. An MB molecule in the lowest triplet state is excited to the higher triplet state by photon absorption, followed by relaxation to the lowest triplet state in its lifetime, that is, the order of $10^{-11}-10^{-12}$ sec; it may then absorb another photon. According to the repetitive process, the apparent lifetime of the excited triplet state is extended to $10^{-9}-10^{-10}$ sec at a high intensity of laser light.

When p-CPG was added to the MB solution as a reducing agent, the photoreduction of MB was observed by means of laser-light irradiation. Flash photolysis studies[7-9] have shown that the photoreduction of MB is accomplished through the lowest triplet state of MB. Therefore, the reaction scheme may be shown as follows:



where S_0, S_1, and T_1 are the ground state, the excited singlet state, and the lowest triplet state of MB respectively. According to this mechanism, the following equation is obtained:

$$\frac{1}{A}=\frac{1}{T}\left\{\frac{k_s}{k_1}\cdot\frac{1}{[p\text{-CPG}]}+\left(1+\frac{k_2}{k_1}\right)\right\} \tag{1}$$

where A is the quantity of MB reduced and T is total amount of MB converted to the triplet state. The plots of $1/A$ against $1/[p\text{-CPG}]$ fall on straight lines at various laser-light intensities, as is shown in Figs. 1 and 2 at 25 and 50°C respectively.

Equation (1) also indicates that the ratio of the intercept to the slope $(k_1+k_2)/k_s$ should be independent of the light intensity. Table 2 shows the values of $(k_1+k_2)/k_s$ for p-CPG, together with those for DMPG.[10] The values obtained with ordinary light[2] are also included. At a low intensity, this ratio is $1.1\times10^5\text{M}^{-1}$ for 8 mJ at 25°C and $4.7\times10^4\text{M}^{-1}$ for 23 mJ at 50°C.

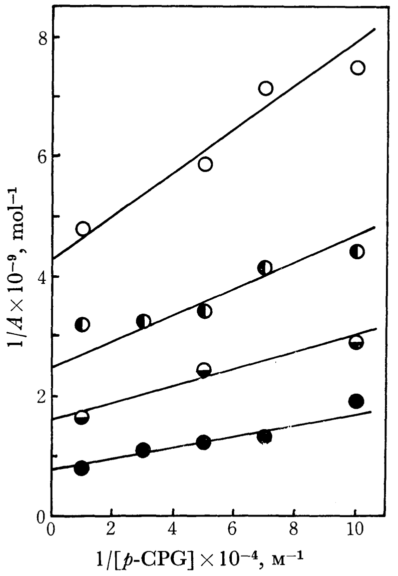


Fig. 1. The reciprocal of the reduced MB *vs.* the reciprocal of the concentration of p-CPG at 25°C.
● 530 mJ, ⊖ 66 mJ, ◐ 23 mJ, ○ 8 mJ.

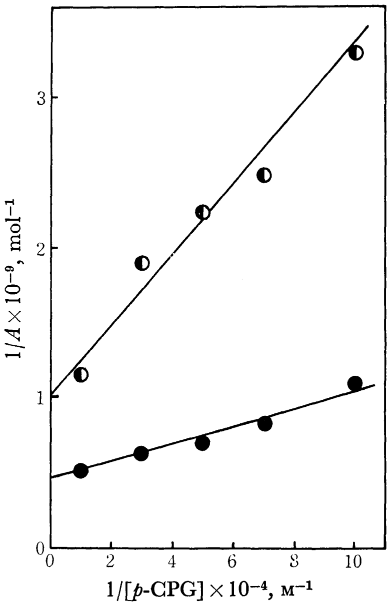


Fig. 2. The reciprocal of the reduced MB *vs.* the reciprocal of the concentration of p-CPG at 50°C.
● 530 mJ, ◐ 23 mJ.

TABLE 2. THE RATIO OF $(k_1+k_2)/k_s$ FOR p-CPG AND DMPG

p-CPG		
Laser intensity	25°	50°
530 mJ	$1.4\times10^5\text{M}^{-1}$	$8.1\times10^4\text{M}^{-1}$
66	1.3	
23	1.2	4.7
8	1.1	
Ordinary light[a]		40° $3.99\times10^4\text{M}^{-1}$
DMPG		
Laser intensity		25°
101 mJ		$14\times10^4\text{M}^{-1}$
45		5.7
4.4		2.8
Ordinary light[a]		40° $3.37\times10^4\text{M}^{-1}$

a) See Ref. 2

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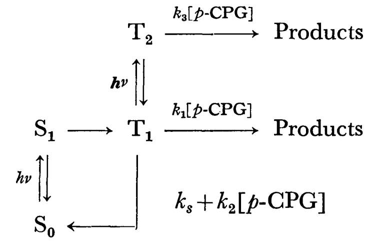
[13] H. Merkelo, S. R. Hartman, T. Mar, and G. S. S. Govindjee, *Science*, **164**, 301 (1969).

[14] R. M. Danziger, K. H. Bar-Eli, and K. Weiss, *J. Phys. Chem.*, **71**, 2633 (1967).

These values are compared with the value for the ordinary light given by Matsumoto.[2] However, this ratio increases with the dose intensity; in case of *p*-CPG, increases of 30 and 70% are observed with an increase in the dose energy from the lowest values to 530 mJ at 25 and 50°C respectively. These values are much smaller than that for DNPG, for which a five-fold increase was observed with the increase in the energy from 4.4 to 101 mJ.

In case of DMPG, the increase in the $(k_1+k_2)/k_s$ ratio with the laser-light intensity is interpreted as the result of the contribution of MB in the excited triplet state.[10] If this is the case for *p*-CPG, the reaction scheme can tentatively be written as follows:



According to the above mechanism, the following equation is obtained:

$$\frac{1}{A}=\frac{1}{T(1+a)}\left\{\frac{k_s}{k_1+bk_3}\,\frac{1}{[p\text{-CPG}]}+\left(1+\frac{k_2}{k_1+bk_3}\right)\right\} \quad (2)$$

where $a=[T_2]/[T_1]$ and $b=\int[T_2]\,dt/\int[T_1]\,dt$. At a low intensity, $a\to0$, $b\to0$, and Eq. (2) is identical with Eq. (1). From Eq. (2), it can be understood why the ratio of the intercept to the slope increases with the dose intensity.

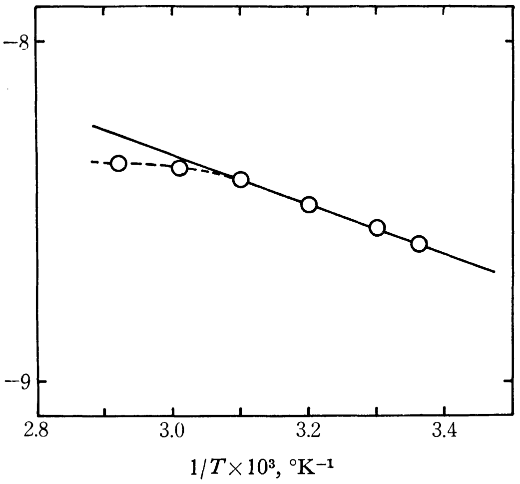


Fig. 3. The typical Arrhenius plot of the reduced MB at 530 mJ. The concentration of *p*-CPG is 10^{-4} M.

The photoreduction of MB was carried out at a rising temperature. Figure 3 shows a typical Arrhenius plot of the quantity of the reduced MB at the dose intensity of 530 mJ. At temperatures lower than 50°C, the plots give a straight line. The apparent activation energy is obtained from the straight part of the Arrhenius plots. It is not clear why the curve deviates from straight line in the temperature region higher than 50°C. The apparent activation energy of the reduction of MB was obtained by varying the laser-light intensity. The results are shown in Fig. 4. The curve shows a decline with an increase in the dose energy up to 270 mJ, while it becomes flat at energy higher than 270 mJ.

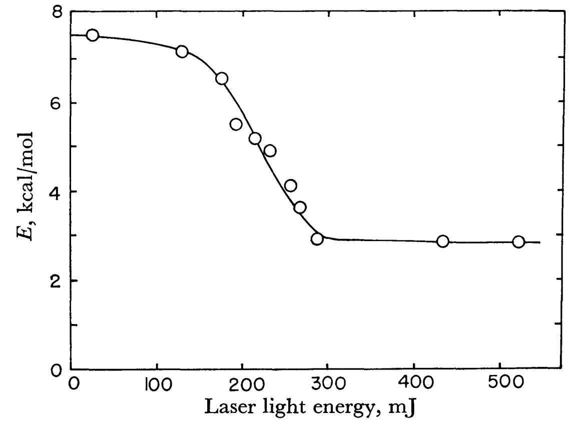


Fig. 4. The apparent activation energy *vs*. the laser light intensity. The concentration of *p*-CPG is 10^{-4}M.

It is supposed that the photoreductions through both the lowest triplet state and the excited triplet state contribute to the apparent activation energy. The activation energy extrapolated to zero light intensity should correspond to the activation energy for the lowest triplet state; it is estimated to be 7.4 kcal mol^{-1}. Since the apparent activation energy of the reaction through the excited triplet state is expected to be lower than that for the lowest triplet state, the decline in the activation energy with the laser intensity is thought to result from the contribution of the reduction of MB through the excited triplet state.

At energy higher than 270 mJ, the apparent activation energy is constant. This can be interpreted by the mechanism in which the reduction mainly occurs through the excited triplet state. Thus, the activation energy in this region would correspond to that of the reduction through the excited triplet state; it can be estimated from Fig. 4 to be 2.8 kcal mol^{-1}.

It is interesting to ascertain the nature of T_2, which was tentatively called the excited triplet state in the previous discussion. As has been mentioned above, the effective lifetime of T_2 is estimated to be 10^{-9}—10^{-10} sec in the absence of the reducing agent at the high-laser intensity, while it is estimated from the diffusion theory that a MB molecule collides with *p*-CPG molecules every 1.4×10^{-6} sec when the concentration of *p*-CPG is 10^{-4} M. This interval between collisions is much longer than the effective lifetime of the excited triplet state. Provided the photoreduction occurs as a result of direct interaction between the excited triplet states of MB and *p*-CPG, the reactionary radii must be about 100 times larger than the diffusional radii. However, if a molecular complex is formed between MB in the lowest triplet state and *p*-CPG, the MB in the excited triplet state may have a better chance to encounter *p*-CPG than would be expected from the simple diffusion theory. This may be the reason why the photoreduction occurs during the short effective lifetime of the excited triplet state.

An alternative interpretation of the nature of T_2 is as follows. When the MB molecule in the excited triplet state relaxes to the lowest triplet state, the electronic excitation energy is consumed in heating the solvent around the MB molecule. If the rate of the heat dissipation is slower than the rate of the heat formation by relaxation, the solvent around the MB molecule is locally heated by the repetitive photo-absorption: thus, the activation energy apparently decreases with a rise in the temperature. Since the apparent activation energy decreases from 7.4 to 2.8 kcal with an increase in the dose intensity, the average temperature of the reaction site is estimated to be 520°C.

At the present stage, it is not possible to determine which mechanism is operative in the photoreduction of MB. However, the long-range interaction proposed in the former case seems to be unreasonable in this case. Thus, we may suppose that the complex mechanism or the temperature effect is responsible for the reduction of MB.

The authors wish to express their thanks to Professors Ikuzo Tanaka and Yuji Mori for their helpful advice and support during the course of this work. They are also grateful to Professor Shiro Matsumoto for help in obtaining the compound used in this study.