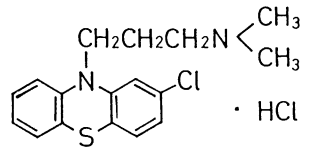
Mechanism of Production of Singlet Oxygen on Photoexcitation of Drugs Inducing Photosensitivity

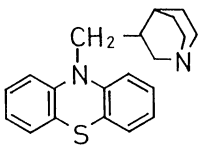
Tatsuo ARAI, Yoshinobu NISHIMURA, Masako SASAKI,*,† Hitoshi FUJITA,††
Itsuro MATSUO,†† Hirochika SAKURAGI, and Katsumi TOKUMARU*
Department of Chemistry, University of Tsukuba, Ibaraki 305
† Institute of Research and Development, Tokai University,
Hiratsuka, Kanagawa 259-12
†† Tokai University, School of Medicine, Isehara, Kanagawa 259-11
(Received December 25, 1990)

The mechanism and active species of drug-induced photosensitive reactions were investigated by measuring the absorption spectra of the transient species on photoexcitation as well as by determining the efficiency of singlet oxygen production on quenching by oxygen of the excited state of drugs. The quantum yields of singlet oxygen production were determined for chlorpromazine hydrochloride, mequitazine, and afloqualone as 0.27, 0.28, and 0.14, respectively. These rather high yields of singlet oxygen suggest its important role in photosensitivity of the drugs. On the basis of the transient-spectroscopic investigations the quenching mechanism of excited-state drugs by oxygen was discussed.

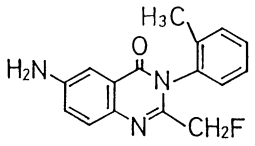
Much attention has been focused on the real active species in photosensitive reactions of drugs. Among drugs benoxaprofen[1] and chlorpromazine[2-6] are reported to undergo photodecarboxylation and photodechlorination, respectively, yielding radical species which can add to DNA or cause disruption of cell membrane. Furthermore, it was reported that benoxaprofen[1b] and chlorpromazine[7] produce singlet oxygen while piroxicam[8,9] produces no detectable amount of singlet oxygen on photoexcitation. In this respect, we investigated the mechanism and active species of drug-induced photosensitive reactions by measuring the absorption spectra of the transient species on photoexcitation and by determining the efficiency of singlet oxygen production on quenching by oxygen of the excited state of drugs which are shown in Chart 1. Among these drugs chlorpromazine hydrochloride (CPZ), mequitazine (MQZ), afloqualone (AQ), and piroxicam (PRX) are known to induce skin photosensitivity, and 5- (5-MOP) and 8-methoxypsoralen (8-MOP) are phototoxic.



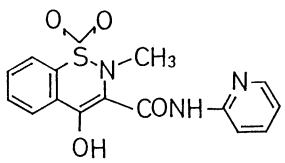
chlorpromazine (CPZ)



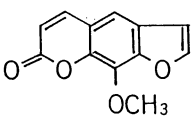
mequitazine (MQZ)



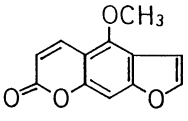
afloqualone (AQ)



piroxicam (PRX)



8-methoxypsoralen (8-MOP)



5-methoxypsoralen (5-MOP)

Chart 1.

Experimental

Materials. CPZ was supplied by Sionogi & Co., MQZ by Nippon Shoji Co., AQ by Tanabe Seiyaku Co., PRX by Pfizer Taito Co., 8-MOP by Tokyo Chemical Industry Co. 5-MOP was a gift from Kanebo Cosmetic Laboratory. These drugs were used as received. Solvent benzene (Luminazol) was obtained from Dojin Chem Co.

Laser Flash Photolyses. Laser flash photolyses and singlet oxygen detections were performed as described previously[10,11] by using 308-nm laser pulses of 10-ns fwhm from an excimer laser (Lambda Physik EMG 101, XeCl) as an exciting light source.

The efficiency of the singlet oxygen production was determined as follows. The decay curves of singlet oxygen ($^1\Delta_g$) emission at 1.27 μm were measured for the solutions optically matched at 308 nm for drugs and benzophenone using a germanium diode as a detector.[10-12] The initial intensities of the singlet oxygen emission were obtained from the decay curves, and the quantum yields for singlet oxygen production (Φ_{SO}) were obtained by comparing the initial intensities with that of benzophenone (BP, Φ_{SO}=0.29) as a standard.[13] The detection limit of the present instrument is 0.02 as the quantum yield of singlet oxygen production.

Results

Determination of the Singlet Oxygen Yields. Figure 1a shows a decay profile of the near-infrared emission of singlet oxygen on excitation of benzophenone in benzene at ambient temperature by a 308-nm laser pulse. Excitation of either CPZ, MQZ, or AQ afforded a similar decay curve. The decay curve observed for MQZ is shown in Figure 1b as an example. The singlet oxygen lifetime (28—32 μs) thus obtained is in good agreement with the value reported.[13] The quantum yields of singlet oxygen production (Φ_{SO}) from the drugs are

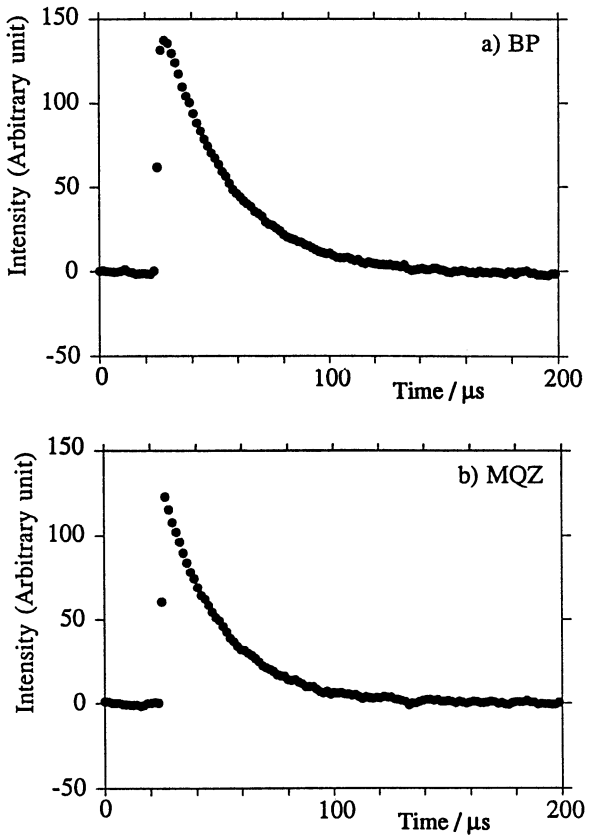


Fig. 1. Decay profiles of singlet oxygen luminescence at 1.27μm in the presence of BP (a) or MQZ (b) in aerated benzene.

obtained using the initial intensities of the emission compared with that of BP (0.29).[13] The quantum yields for CPZ, MQZ, and AQ are 0.27, 0.28, and 0.14, respectively. However, the emission could not be detected for PRX,[9] 5-MOP, and 8-MOP under our experimental conditions, indicating that Φ_{SO} for these compounds must be lower than 0.02. These low values for 5-MOP and 8-MOP are in accordance with the values reported.[14]

T_1-T_n Absorption Spectra and Their Quenching Rate Constants by Oxygen. Figure 2 shows the transient absorption spectra of CPZ, MQZ, and AQ in benzene. CPZ exhibited its transient spectrum at 360—700 nm (λ_{max}: 480 nm) which decayed by first-order kinetics (τ_T=5.8 μs) under argon atmosphere and was efficiently quenched by oxygen (k_q=1.4×10^{10} M^{-1} s^{-1}, M=mol dm^{-3}). The maximum wavelength in the present observation in benzene is shifted to the longer wavelength compared with that reported[15,16] in mixed solvents of alcohols and water (λ_{max}: 460—465 nm). The observed lifetime and the oxygen quenching rate constant suggest that this spectrum is attributed to the T_1–T_n absorption. MQZ also exhibited the T_1–T_n absorption spectrum at 360—700 nm (λ_{max}: 480 nm, τ_T=4.2 μs) and was quenched by oxygen (k_q=1.6×10^{10} M^{-1} s^{-1}). AQ exhibited an intense

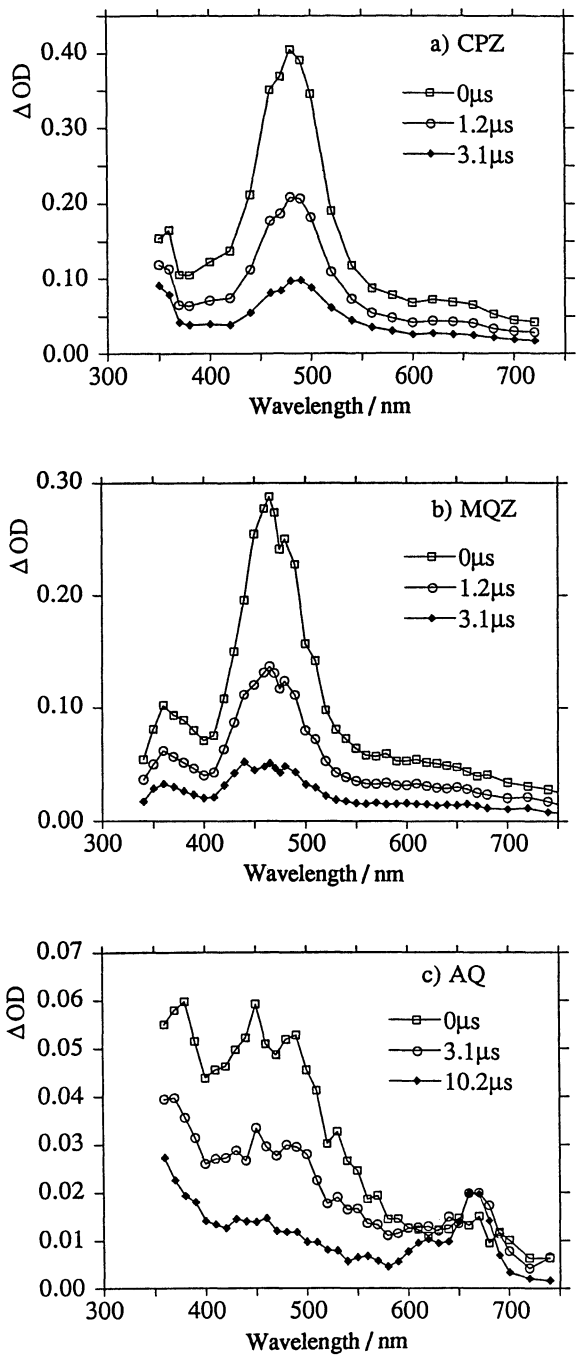


Fig. 2. Transient absorption spectra observed on 308 nm excitation of CPZ (a), MQZ (b), and AQ (c) in benzene.

absorption band at 360—600 nm (λ_{max}: 450 nm) accompanied by a weak band at 600—720 nm; the former band decayed with a lifetime of 6.7 μs (Fig. 3a and b) under argon and was quenched by oxygen at a nearly diffusion-controlled rate (k_q=6.4×10^9 M^{-1} s^{-1}), and the latter band around 670 nm increased in intensity with the μs time scale (Fig. 3c). This behavior indicates occurrence of chemical reactions at the excited state of AQ.

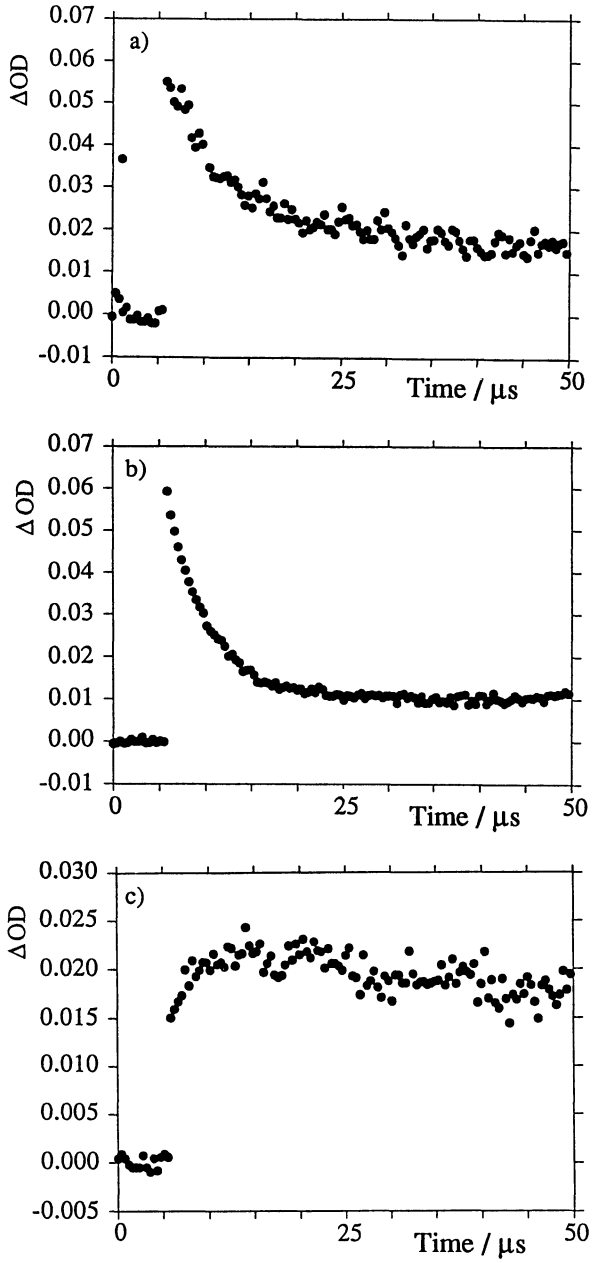


Fig. 3. Time profiles of transient absorptions monitored at 360 nm (a), 450 nm (b), and 670 nm (c) following 308 nm laser photolysis of AQ in benzene.

Discussion

Possible Quenching Mechanism by Oxygen. The quenching of excited triplet states by oxygen proceeds as shown by Eqs. 1—3, where spin statistical factors in triplet-triplet annihilation play an important role. If the triplet state of a substrate has an energy higher than that of singlet oxygen, 95 kJ mol^{-1}, the quenching proceeds through energy transfer (Eq. 1). The probability for this process is only 1/9 for every collisional encounter, and thus, the rate constant for this process must be 1/9 of the diffusion-controlled rate constant (3×10^9 M^{-1} s^{-1}).[17] On the other hand, quenching by acceleration of intersystem crossing to the ground state (Eq. 2) becomes more important as the energy difference between the ground S_0 and triplet T_1 states becomes smaller than 95 kJ mol^{-1}. The triplet energies of CPZ, MQZ, AQ, 5-MOP, and 8-MOP were determined as 246, 247, 238, 252, and 259 kJ mol^{-1}, respectively, by phosphorescence measurements.[18] From these values the oxygen quenching is presumed to proceed through energy transfer (Eq. 1).

$$^3S^*+{}^3O_2 \underset{}{\overset{k_d/9}{\rightleftarrows}} {}^1[S\cdots O_2]^* \longrightarrow {}^1S+{}^1O_2^* \quad (1)$$

$$\overset{3k_d/9}{\rightleftarrows} {}^3[S\cdots O_2]^* \longrightarrow {}^1S+{}^3O_2 \quad (2)$$

$$\overset{5k_d/9}{\rightleftarrows} {}^5[S\cdots O_2]^* \quad (3)$$

Mechanism of Singlet Oxygen Production.

$$S \xrightarrow{h\nu} {}^1S^* \quad (4)$$

$$^1S^* \xrightarrow{k_5} S \quad (5)$$

$$^1S^* \xrightarrow{k_6} S+h\nu \quad (6)$$

$$^1S^* \xrightarrow{k_7} {}^3S^* \quad (7)$$

$$^1S^*+O_2 \xrightarrow{k_8} {}^3S^*+{}^1O_2^* \quad (8)$$

$$^1S^*+O_2 \xrightarrow{k_9} {}^3S^*+O_2 \quad (9)$$

$$^3S^*+O_2 \xrightarrow{k_{10}} S+{}^1O_2^* \quad (10)$$

$$^3S^*+O_2 \xrightarrow{k_{11}} S+O_2 \quad (11)$$

$$^3S^* \xrightarrow{k_{12}} S \quad (12)$$

$$\Phi_{SO}=\frac{\{k_8[O_2]+\dfrac{k_{10}[O_2]\{k_7+(k_8+k_9)[O_2]\}}{\{(k_{10}+k_{11})[O_2]+k_{12}\}}\}}{\{k_5+k_6+k_7+(k_8+k_9)[O_2]\}} \quad (13)$$

$$\Phi_{SO}=k_7k_{10}/(k_5+k_6+k_7)(k_{10}+k_{11})=\Phi_{isc}\Phi_\Delta \quad (14)$$

The quenching of excited drugs by oxygen should take place as shown in Eqs. 4—12, where S denotes a drug. The quantum yield of singlet oxygen production (Φ_{SO}) is described as Eq. 13. If the quenching of excited singlet drugs by oxygen is not important ($(k_5+k_6+k_7)\gg$

$(k_8+k_9)[O_2]$), Φ_{SO} can be reduced to Eq. 14, where Φ_{isc} $(=k_7/(k_5+k_6+k_7))$ and Φ_Δ $(=k_{10}/(k_{10}+k_{11}))$ mean the quantum yields of intersystem crossing from the singlet excited states to the triplet excited states and the efficiency of singlet oxygen production in the quenching of the triplet molecules by oxygen, respectively.

Therefore, the efficiency of singlet oxygen production is related to the quantum yield of intersystem crossing of the excited singlet state of drugs. The quantum yields of intersystem crossing were reported to be as low as 0.067 and 0.011[19] for 5-MOP and 8-MOP, respectively. Thus, these low Φ_{ISC} values might be the reason why no singlet oxygen emission was detected from 5-MOP and 8-MOP. Actually, Φ_{SO}'s are reported as 0.021 and 0.0044 for 5- and 8-MOP,[14] respectively, which are below our detection limit.

Quenching of a singlet excited state by oxygen can give directly singlet oxygen together with the triplet excited state, if the energy difference between the singlet and triplet excited states is higher than the excitation energy of singlet oxygen (95 kJ mol^{-1}). However, this is not the case since the energies of the singlet excited state of CPZ and AQ are measured as 271 and 276 kJ mol^{-1},[18] respectively, and accordingly, they cannot produce singlet oxygen on their quenching by oxygen (E_T: 246 and 238 kJ mol^{-1} for CPZ and AQ, respectively).[18]

Therefore, the efficiency of singlet oxygen production observed for the drugs examined mainly reflects those of intersystem crossing and singlet oxygen production in the quenching of their triplet state. The quenching of the triplet molecules having enough energies to produce singlet oxygen is usually assumed to produce singlet oxygen with a nearly unit efficiency, and thus, we can tentatively assume the efficiency of singlet oxygen production as a lower limit of the intersystem crossing efficiency for these drugs.

The observed quenching rate constants for ^{3}CPZ*, ^{3}MQZ*, and ^{3}AQ* by oxygen are 2—5 times larger than the assumed value (3×10^9 M^{-1} s^{-1}) from Eq. 1. Therefore, there must be some other interactions resulting in the efficient production of singlet oxygen.

For some molecules, participation of the electron transfer mechanism[20] is proposed in the quenching of their triplet states by oxygen. For example, the observed rate constats of oxygen quenching for substituted benzophenone triplets exceed a limit imposed by the spin-statistical restriction (3×10^9 M^{-1} s^{-1}); the quenching rate constant for 4,4′-bis(dimethylamino)benzophenone (DAB, $k_q=12.3\times10^9$ M^{-1} s^{-1}) is more than 5 times higher than that for benzophenone (2.3×10^9 M^{-1} s^{-1}). However, the singlet oxygen is produced with the same efficiency in the quenching of triplet DAB as from triplet benzophenone. The aforementioned large rate constants determined for the present drugs might suggest an intervention of the electron transfer interaction in addition to the usual energy transfer mechanism in the quenching process to produce singlet oxygen.

It should be noted that singlet oxygen ($^1\Delta_g$) is often claimed as an acting species of photosensitivity of drugs based only on the observation of their triplet states and their quenching by oxygen. However, as has been revealed recently, the efficiency of singlet oxygen production is very much dependent on the properties of the triplet excited state. For example, aromatic ketones such as benzophenone produce singlet oxygen not with a unit efficiency but with a rather low value, 0.29.[13] In this case the quenching is supposed to proceed not only by energy transfer but also by addition of oxygen on the carbonyl moiety producing biradical species.[21] Measurements of intersystem crossing yields of durgs will be useful to elucidate the quenching mechanism and the relation between quenching rates and singlet oxygen yields. However, the present determination of the singlet oxygen yields by the direct observation of emission gives an important information for the elucidation of real active species in the photosensitive reactions of drugs.

Conclusion

Quenching of the triplet state of CPZ, MQZ, and AQ by oxygen efficiently produces singlet oxygen, which seems to be responsible for the photosensitive reactions induced by these drugs. Furthermore, AQ also undergoes photoreactions even without oxygen, and can cause additional photosensitive reactions.

The authors thank the Ministry of Education, Science and Culture for partial support by Grant-in-Aid for Special Project Research Nos. 61123001, 62113001, and 63104001 (KT), Nos. 61223023, 62213029, and 63104001 (MS), and by Grant-in-Aid for Scientific Research No. 01740255 (TA).

References

1) a) K. Reszka and C. F. Chignell, *Photochem. Photobiol.*, **38**, 281 (1983); b) S. J. Navaratnam, L. Hughes, B. J. Parsons, and G. O. Phillips, *Photochem. Photobiol.*, **41**, 375 (1985).

2) F. Laterrier, A. Mendyk, and J. Viret, *Biochem. Pharmacol.*, **25**, 2469 (1976).

3) I. E. Kochevar, *J. Invest. Dermatol.*, **76**, 59 (1981).

4) H. Fujita, F. Yanagisawa, A. Endo, and K. Suzuki, *J. Radiat. Res.*, **21**, 279 (1980).

5) G. Testylier, G. Clement, and F. Leterrier, *Photochem. Photobiol.*, **39**, 277 (1984).

6) A. G. Motten, G. R. Buttener, and C. Chignell, *Photochem. Photobiol.*, **42**, 9 (1985).

7) R. D. Hall, G. R. Buettner, A. G. Motten, and C. F. Chignell, *Photochem. photobiol.*, **46**, 295 (1987).

8) I. E. Kochevar, W. L. Morison, J. L. Lamm, D. J. Mc Auliffe, A. Wester, and A. F. Hood, *Arch. Dermatol.*, **122**, 1283 (1986).

9) A. Western, J. R. Van Camp, R. Bensasson, E. J. Land, and I. E. Kochevar, *Photochem. Photobiol.*, **46**, 469 (1987).

10) T. Arai, T. Karatsu, M. Tsuchiya, H. Sakuragi, and K. Tokumaru, *Chem. Phys. Lett.*, **149**, 161 (1988).

11) H. Okamoto, T. Arai, H. Sakuragi, and K. Tokumaru, *Bull. Chem. Soc. Jpn.*, **63**, 2881 (1990).
12) M. A. J. Rodgers and P. T. Snowden, *J. Am. Chem. Soc.*, **104**, 5541 (1982).
13) A. A. Gorman, I. Hamblett, and M. A. J. Rodgers, *J. Am. Chem. Soc.*, **106**, 4679 (1984).
14) C. N. Knox, E. J. Land, and T. G. Truscott, *Photochem. Photobiol.*, **43**, 359 (1986).
15) T. Iwaoka and M. Kondo, *Bull. Chem. Soc. Jpn.*, **50**, 1 (1977).
16) S. B. Navaratnam, J. Parsons, and G. O. Phillips, *J. Chem. Soc., Faraday Trans. 1*, **74**, 1811 (1978).
17) O. L. Gijzeman, J. F. Kaufman, and G. Porter, *J. Chem. Soc., Faraday Trans. 2*, **69**, 708 (1973).
18) M. Sasaki, H. Fujita, and I. Matsuo, unpublished results.
19) R. V. Bensasson, E. J. Land, and C. Salet, *Photochem. Photobiol.*, **27**, 273 (1978).
20) S. K. Chattopadhyay, C. V. Kumar, and P. K. Das, *J. Photochem.*, **30**, 81 (1985).
21) A. A. Gorman and M. A. J. Rodgers, *J. Am. Chem. Soc.*, **108**, 5074 (1986).