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Mechanism of Dye-sensitized Photooxidation of Tryptophan, Tryptamine, and Their Derivatives. Singlet Oxygen Process in Competition with Type I Process

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Photosensitized oxidations of tryptophan (Trp), tryptamine, and their derivatives were investigated with a variety of sensitizers. Rose Bengal sensitized photooxidations proceed exclusively *via* Type II mechanism (singlet oxygen mechanism), whereas for thionine-sensitized photooxidations both Type I (nonsinglet oxygen mechanisms) and Type II mechanisms are operative under a given set of conditions. Rate constants of reaction, in units of 10^6 M^{-1} s^{-1}, were determined to be 3 for Trp, 4 for tryptophan methyl ester, 4 for N_b-acetyltryptophan methyl ester, 9 for tryptamine, and 7 for N_b-methoxycarbonyltryptamine in the Rose Bengal-sensitized photooxidations. These values are in good agreement with those obtained from reactions with chemically generated singlet oxygen. Quenching rate constants are somewhat larger than reaction rates constants, indicating that the quenching process is the major path for the interaction of singlet oxygen with these indolic substrates. Oxidation products in chemically generated singlet-oxygen reaction of Trp are compared with those obtained from dye-sensitized photooxidations.

Photooxidation of tryptophan (Trp) is of particular interest in connection with photodynamic degradation of Trp residues in proteins, and the possibility that the oxidation may represent a model for biological oxidation of Trp catalyzed dioxygenases. A number of reports on dye-sensitized photooxidation of Trp and related substrates have appeared in connection with photoproducts as well as mechanisms. The photooxidation of Trp gives many types of products, depending on experimental conditions such as wavelength of light, type and concentration of sensitizers, and solvents employed.[1] In earlier papers,[2] the photosensitized oxidation of Trp was shown to give a complex mixture of products, in which kynurenine and *N*-formylkynurenine (*N*-FK) were detected. Savige[3] reported that Methylene Bule (MB)-sensitized photooxidation of Trp in water gave *N*-FK and 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acid (HPI). More recently, Nakagawa *et al.*[4] have reported isolation of 3a-hydroperoxy-1,2,3,3a,8,8a-hexahydropyrrole[2,3-*b*]indole-2-carboxylic acid (HPPI) as a primary product in Rose Bengal (RB)-sensitized photooxidation. This hydroperoxide (HPPI) decomposes to give *N*-FK and HPI under photolytic or thermolytic conditions.[4]

The mechanism of dye-sensitized photooxidation of Trp has long been a subject of controversy. Trp is reported to quench triplet states of sensitizers such as eosine and thionine under certain conditions.[5] A recent work[6] has demonstrated that the excited singlet states of RB and MB are also quenched by tryptamine and other indoles. These results may imply the importance of a nonsinglet oxygen (Type I[7]) mechanism involving interaction between photoexcited dyes and indoles in the sensitized photooxidation of Trp. In contrast to these reports, Nilsson *et al.*[8] pointed out that singlet oxygen ($^1O_2$) is involved in MB-sensitized photooxidation of Trp in aqueous methanol, and they have determined rate constants for the reaction (k_r) and quenching (k_q) of Trp with $^1O_2$. However, the rate constant of reaction, k_r, reported by Nilsson *et al.*[8] is approximately one order of magnitude smaller than the value obtained from the reaction of Trp with photophysically generated $^1O_2$ in D_2O (pD 8.4),[9] although the overall rate constant, k_r+k_q, has the same order of magnitude. Smith[10] showed that rate constants are highly dependent on the polarity of solvent. Furthermore, recent works[11,12] on photooxidations of Trp and tryptamine with hematoporphyrin sensitizer have shown that the reactions proceed *via* Type I mechanism, and that the singlet oxygen (Type II) mechanism is of minor importance even when reactions are carried out in the presence of micelles. These results apparently indicate that the dye-sensitized photooxidations of Trp and tryptamine are strongly dependent on reaction conditions, particularly on the type of sensitizer, solvent, and substrate concentration.

In this paper, the influence of conventional sensitizers such as RB and MB on the mechanism of sensitized photooxidation of Trp, tryptamine, and their derivatives has been examined as a model for photooxidation of Trp residues in a protein. Rate constants for the reaction and quenching of these substrates with $^1O_2$ in the RB-sensitized photooxidation have been compared with those obtained with chemically generated $^1O_2$. Oxidation products in both reactions were also examined.

Experimental

Materials. Rose Bengal (RB), Methylene Blue (MB), eosine, thionine, L-tryptophan (Trp, **1**), tryptophan methyl ester (**2**), and tryptamine (**4**) are commercially available. N_b-Acetyltryptophan methyl ester (**3**) and N_b-methoxycarbonyltryptamine (**5**) were prepared by the known methods. 3-(1,4-Epidioxy-4-methyl-1,4-dihydro-1-naphthyl)propionic acid (EP) was prepared by the published procedure.[13]

Procedure. A typical irradiation system consists of 10 ml of 1:1 MeOH–H_2O solution containing **1** (1.3×10^{-4} M) (1 M = 1 mol dm^{-3}), sensitizer (3.3×10^{-6} M), and varying amounts of sodium azide ($2—50\times10^{-3}$ M) in Pyrex reaction tubes. After the solutions were saturated with pure oxygen, the test tubes were sealed with jointed stoppers.

Table 1. Effects of azide ion and deuterated solvent on the rates of photooxidations of tryptophan derivatives with various sensitizers[a]

Sensitizer	R = -CH(CO$_2$H)-NH$_2$ (1)		R = -CH(CO$_2$Me)-NH$_2$ (2)		R = -CH(CO$_2$Me)-NHCOMe (3)		R = -CH$_2$NH$_2$ (4)		R = -CH$_2$NHCO$_2$Me (5)	
	I/%[b]	R_D/R_H[c]	I/%[b]	R_D/R_H[c]	I/%[b]	R_D/R_H[c]	I/%[b]	R_D/R_H[c]	I/%[b]	R_D/R_H[c]
Rose Bengal (RB)	100	9.6 (8.1)[d]	100	9.8 (8.7)[d]	100	10.2 (8.9)[d]	100	7.4	100	11.4 (9.5)[d]
Methylene Blue (MB)	90	9.0 (8.7)[d]	90	11.5 (9.1)[d]	90	9.5 (8.5)[d]	90	7.4	90	8.0 (6.5)[d]
Eosine	100	11.8	90	8.7	100	8.2	80	7.0	100	9.8
Thionine	60	7.5	80	8.3	80	8.3	40	4.0	40	4.7

a) In 1:1 MeOH-H_2O; [sens]$=3.3\times10^{-6}$ M; [substrate]$=1.3\times10^{-4}$ M. b) I denotes $(R_0-R_1)/R_0\times100$; R_0: the rate of photooxidation in the absence of NaN_3; R_1: the limiting rate of photooxidation in the presence of higher concentrations of NaN_3. c) The ratio of the photooxidation rates in 1:1 MeOD-D_2O *vs.* 1:1 MeOH-H_2O. d) The values in parentheses are for the higher concentrations of substrate; [substrate]$=3.7\times10^{-3}$ M.

The samples were irradiated with a 500 W tungsten-bromine lamp operated at 40 V in a merry-go-round apparatus. Rates of photooxidation of **1** were followed by monitoring the decrease in the absorption maximum at 280 nm with a Nihon Bunko UV-1 spectrometer.

Measurement of Photooxidation Rates in MeOD-D_2O and in MeOH-H_2O. A comparison of photooxidation rates in 1:1 MeOD-D_2O and in 1:1 MeOH-H_2O was done in a merry-go-round apparatus under identical conditions. Rates of photooxidation were measured by the method described above.

Determination of Rate Constants. Rate constants for the reaction, k_r, and quenching, k_q, were measured by the direct disappearance method described by Foote and Ching[14] and by the competition technique. Solutions of various concentrations ($(0.3—3)\times10^{-4}$) of **1** containing RB (4×10^{-6} M) were irradiated. The concentrations of **1** before and after irradiation were determined from decrease in its absorption maximum. The relationship between the reciprocal of the amount of reacted **1** and the reciprocal of the initial concentration of **1** gives a straight line. From the slope and intercept, the sum, k_r+k_q, of reaction and quenching rate constants was obtained (within 10% error). Rate constants of the reactions were obtained from competition reactions. To a MeOH-H_2O solution of **1** containing RB was added a MeOH solution of diphenylisobenzofuran (DPBF), and the solution was irradiated as described above. Periodical change in concentration of **1** and DPBF was measured by decrease in the absorption maximum of **1** and in the fluorescence intensity of DPBF. Similar experiments were performed in order to determine in rate constant for **1** with $^1O_2$ generated from endoperoxide (EP). A solution of **1** (3.5 mM) and EP (25 mM) was warmed up to 35 °C with shaking. The rate constant was calculated from the disappearance of the peak area for **1** in high-performance liquid chromatography (HPLC, Waters, ALC/GPC 204, a Nucleosil $5C_{18}$ column, 90% aqueous methanol).

Quenching of Excited Singlet State of Dye. The fluorescence of dye was measured in the absence and presence of substrates **1**—**5** with a Nihon Bunko FP 550 at ambient temperature.

Results and Discussion

Participation of $^1O_2$ in the RB-sensitized photooxidation of **1**, **2**, **3**, **4**, and **5** was examined by the following experiments: (i) inhibition by azide ion, a well known singlet oxygen ($^1O_2$) quencher with a quenching rate constant of 10^8 M^{-1} s^{-1};[15] (ii) deuterium solvent effect on the rate of their photooxidations.[8]

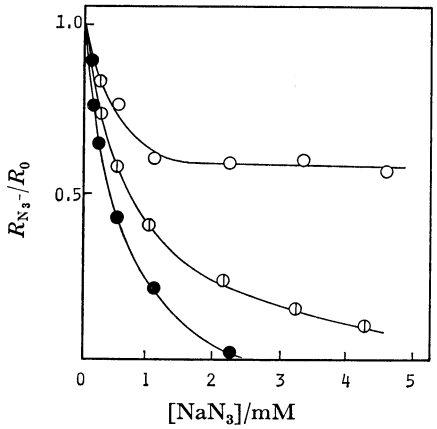


Fig. 1. Sodium azide inhibition of RB-(●), MB-(①), and thionine-(○) sensitized photooxidations of N_b-methoxycarbonyltryptamine (**5**) in methanol-water (1:1) at 25 °C as a function of sodium azide concentration.
[**5**]$=1.6\times10^{-4}$ M; [sens]$=3.3\times10^{-6}$ M.

Inhibition by Azide Ion. Reaction rate $R_{N_3^-}$ of RB-sensitized photooxidation of **5** in the presence of N_3^- was compared with that of control run, R_0. Ratio $R_{N_3^-}/R_0$ decreases with increasing concentration of N_3^- and the photooxidation was completely inhibited at concentration of 2.2 mM of N_3^-. In contrast, methylene blue (MB)- and thionine-sensitized photooxidations of **5** require much higher concentrations of N_3^- to exhibit the same extent of inhibition. In these cases, ratio $R_{N_3^-}/R_0$ decreases with increasing N_3^- concentration and reaches a constant value R_1 at higher concentrations of N_3^- (Fig. 1). By using this limiting value R_1, the extent I of contribution of $^1O_2$ process to overall reaction is calculated from the equation $I=(R_0-R_1)/R_0\times100$. Similar results have been obtained in the photooxidations of **1** and other indolic substrates **2**—**4** as summarized in Table 1. The data in Table 1 suggest that RB-sensitized photooxidations of

Table 2. Quenching of the fluorescence of dye sensitizers with tryptophan and tryptamine derivatives[a)]

Substrate	$k_q^s\tau/M^{-1}$ ($k_q^s/10^{-9}\,M^{-1}\,s^{-1}$)		
	Rose Bengal	Methylene Blue	Eosine
Tryptophan (**1**)	10.5 (16)	22	19 (0.59)
Tryptophan methyl ester (**2**)	7.5 (11)	19	21 (0.65)
N_b-Acetyltryptophan methyl ester (**3**)	15 (23)	23	24 (0.75)
Tryptamine (**4**)	13 (20)	32	23 (0.71)
N_b-Methoxycarbonyl-tryptamine (**5**)	10 (15)	13	25.5 (0.79)

a) Optical density of dye sensitizer is adjusted to 0.1 at the excitation wavelength.

1—**5** proceed exclusively *via* the $^1O_2$ (Type II) mechanism, whereas the lack of complete inhibition by N_3^- in MB- and thionine-sensitized photooxidations indicated a significant contribution of Type I process and/or the quenching of the sensitizer triplets by N_3^-.

Azide ion is known to quench not only $^1O_2$ but also triplet states of certain dye sensitizers. Quenching rate constants $k_q(N_3^-)$ for sensitizer triplets have been determined previously, *e.g.*, $k_q(N_3^-)=5\times10^5\,M^{-1}\,s^{-1}$ for MB[16] and $k_q(N_3^-)=8\times10^5\,M^{-1}\,s^{-1}$ for eosine.[16] These rate constants are much smaller than those for quenching of the triplet states of MB and eosine by oxygen, $k_q(O_2)=3\times10^9\,M^{-1}\,s^{-1}$ and $k_q(O_2)=1.1\times10^9\,M^{-1}\,s^{-1}$,[17] respectively. Therefore, the interaction between triplet sensitizers and N_3^- may be negligibly small in MB- and eosine-sensitized photooxidations. On the other hand, sensitizer triplets can interact with substrates to result in hydrogen abstraction or electron transfer (D-R mechanism).[18] In fact, Trp is known to quench triplet states of sensitizers with the following rate constants: $k_q^t=2\times10^9\,M^{-1}\,s^{-1}$ for MB,[8] $k_q^t=8.4\times10^8\,M^{-1}\,s^{-1}$ for eosine,[5] and $k_q^t=3.9\times10^9\,M^{-1}\,s^{-1}$ for thionine.[5] These rate constants are comparable to the rates of quenching of the respective sensitizer triplets by oxygen. Thus, it seems probable that MB-, eosine-, and thionine-sensitized photooxidations may involve Type I process in which sensitizer triplets react directly with indolic substrates. The inhibitory effect of N_3^- (Table 1) and the deuterium solvent effect on the rate of photooxidation (*vide infra*) indicate that in MB- or eosine-sensitized photooxidation direct reaction of triplet sensitizer with Trp is a minor process with the major process being $^1O_2$ reaction. In thionine-sensitized photooxidation, however, Type I reaction involving the thionine triplet cannot be neglected as judged from the inhibitory effect of N_3^- as shown in Table 1.

In order to get information on the interaction between singlet dye sensitizers and indolic substrates, quenching of the fluorescence of sensitizers by **1**—**5** was examined in MeOH–H_2O. The value $k_q^s\tau$, which is obtained from the slope of Stern-Volmer plots, may be taken as a rough measure for the interaction between singlet state of sensitizer and substrate. From $k_q^s\tau$ values apparent rate constants for the quenching of singlet state of sensitizers were calculated from the known lifetimes of singlet of sensitizers, $\tau=3.2\times10^{-9}$ s for eosine[19] and $\tau=6.5\times10^{-10}$ s for RB.[19] As shown in Table 2, the apparent rate constants are close to a diffusion controlled limit. This result may imply that the interaction between the excited singlet state of sensitizer and substrate becomes more important when higher concentrations of substrate and/or sensitizer are used, as pointed out by Davidson and Trethewey.[6]

Deuterium Solvent Effect on the Rate of Photooxidation. The lifetime of $^1O_2$ is known to be approximately 10 times as long in MeOD–D_2O as in MeOH–H_2O.[8] Therefore, about tenfold increase in the rate of photooxidation might be expected for a pure $^1O_2$ reaction in going from protonated to deuterated solvent.[20] However, this increase would be smaller if Type I mechanism becomes predominant or if the acceptor concentration is high enough to quench $^1O_2$ significantly. The results are shown in Table 1. The rates of RB-sensitized photooxidation of Trp and its derivatives **2** and **3** increase approximately tenfold at lower concentrations of substrates in going from MeOH–H_2O to MeOD–D_2O. The R_D/R_H value for the photooxidation of **4** was somewhat smaller than that expected from the inhibitory effect by N_3^- (Table 1), the reason remaining unknown. However, as will be discussed below, a kinetic treatment indicates that the photooxidation of **4** occurs exclusively *via* the $^1O_2$ mechanism. The deuterium solvent effect together with inhibition experiments clearly demonstrates that RB-sensitized photooxidations of **1**—**5** proceed by the $^1O_2$ mechanism.

For the thionine-sensitized photooxidation, both Type I and $^1O_2$ (Type II) processes are operative under our experimental conditions. The ratio (I) of $^1O_2$ process to overall reaction as obtained from the inhibition experiment is consistent with the R_D/R_H value, suggesting that the lack of complete inhibition by N_3^- in thionine-sensitized photooxidation is mainly due to the contribution of Type I process. It seems probable that the thionine triplet tends to increase the ratio of a free radical process by reacting with indolic substrates directly.[21] In every case, at higher concentrations of substrates R_D/R_H values become smaller, suggesting an increasing contribution of Type I process at higher substrate concentrations (Table 1).

Determination of Rate Constants. Kinetic scheme for the RB-sensitized photooxidation can be written as

$$^1O_2 + A \xrightarrow{k_r} AO_2,$$
$$^1O_2 + A \xrightarrow{k_q} A + O_2,$$
$$^1O_2 \xrightarrow{k_d} O_2,$$

Scheme 1.

where A denotes substrate. An application of the steady-state approximation to $^1O_2$ gives the following expression for the rate of disappearance of A:[14]

$$\frac{d[A]}{dt} = K\left(\frac{k_r[A]}{(k_r+k_q)[A]+k_d}\right)$$

Table 3. Rate constants of tryptophan, tryptamine, and their derivatives with singlet oxygen in methanol-water

Substrate	$^1O_2$ Source	Rate constant/10^7 M^{-1} s^{-1}			Ref.
		k_r+k_q	k_r	k_q	
Tryptophan (**1**)	RB/*hν*/O_2	7.1	0.3	6.8	This work
	EP	2.1	0.5	1.6	This work
	MB/*hν*/O_2	4.4	0.4	4.0	Nilsson *et al.*[8]
	MB/*hν*/O_2	3.0			Smith[a),10]
	Flash photolysis technique	6.1			Rodgers *et al.*[b),24]
	Photophysical method	5.1	3.0	2.1	Matheson *et al.*[c),9]
Tryptophan methyl ester (**2**)	RB/*hν*/O_2	3.9	0.4	3.5	This work
N_b-Acetyltryptophan methyl ester (**3**)	RB/*hν*/O_2	6.3	0.4	5.9	This work
	EP	2.4	0.6	1.8	This work
Tryptamine (**4**)	RB/*hν*/O_2	5.5	0.9	4.6	This work
N_b-Methoxycarbonyl-tryptamine (**5**)	RB/*hν*/O_2	3.2	0.7	2.5	This work
	EP	3.2	1.3	1.9	This work

a) 1:1 MeOH-H_2O. b) In D_2O. c) In D_2O (pD 8.4).

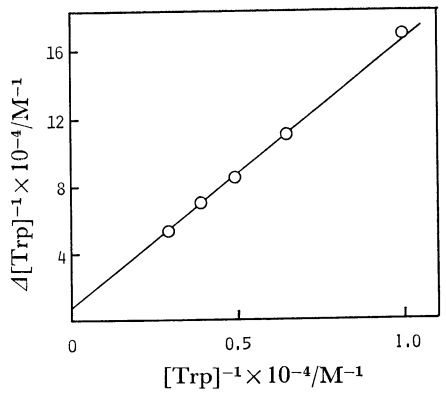


Fig. 2. Double reciprocal plot of tryptophan disappearance *vs.* tryptophan concentrations for the RB-sensitized photooxidation of tryptophan in methanol–water (1:1).
[sens]$=4\times10^{-6}$ M; [**1**]$=0.3$—3×10^{-4} M.

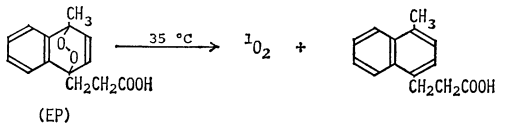
$$\Delta[A]^{-1} = (K\Delta t)^{-1}\left(\frac{k_r+k_q}{k_r}+\frac{k_d}{k_r}[A]^{-1}\right),$$

where K is the rate of $^1O_2$ formation. In fact, plots of $[Trp]^{-1}$ *vs.* $\Delta[Trp]^{-1}$ gave a linear relationship as shown in Fig. 2. For the RB-sensitized photooxidation of **4**, a straight line was also obtained, suggesting that only $^1O_2$ is participating in the reaction. From the slope and intercept, $k_d/(k_r+k_q)$ was obtained and the overall rate k_r+k_q was determined by using the literature k_d value[22] ($k_d=2.8\times10^5$ M^{-1} s^{-1}). In order to determine the absolute rate constants of k_r and k_q, a competition experiment between substrate and diphenylisobenzofuran (DPBF) was carried out. Disappearance rates of **1** and DPBF were determined from decrease in the absorption at 280 nm and in the fluoresence intensity at 477 nm, respectively. The kinetic equation for the competition reaction can be written as

$$\frac{k_r}{k_r^{DPBF}} = \frac{\log([A]_f/[A]_0)}{\log([DPBF]_f/[DPBF]_0)}.$$

The ratio k_r/k_r^{DPBF} obtained for **1** is 3.8×10^{-3}, and k_r was calculated to be 3×10^6 M^{-1} s^{-1} by using $k_r^{DPBF}=8\times10^8$ M^{-1} s^{-1} in 1:1 MeOH-H_2O.[8] The k_r value obtained is in fairly good agreement with that of Nilsson *et al.*[8] However, the rate constant k_r is approximately one order of magnitude smaller than that of Matheson and Lee,[9] although the quenching rate constants are comparable in both cases. The results together with the reported values are summarized in Table 3.

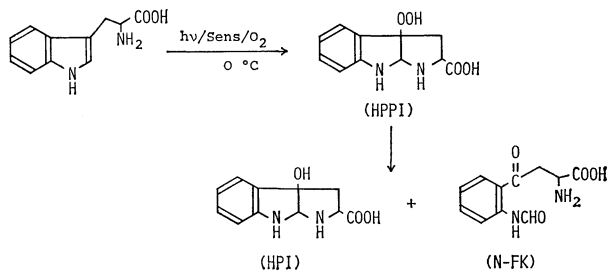
In view of the disagreement of our values with those obtained by Matheson and Lee,[9] we have performed a reaction with chemically generated $^1O_2$ from EP. It is noted that the thermolysis of EP results in nearly quantitative (>82%) production of $^1O_2$ at ambient



Scheme 2.

temperature.[13] The rate constant of reaction of **1** with chemically generated $^1O_2$ was in good agreement with the value obtained in the RB-sensitized photooxidation, whereas the quenching rate constant is somewhat larger than that obtained in the sensitized photooxidation (Table 3). A similar tendency was observed for **3** and **5**. The reason for this discrepancy is not clear. However, it is possible that the difference is due to difficulties in separating Type I and II processes kinetically. Thus substrates **1**—**5** quench $^1O_2$ with a somewhat higher rate than they react, as pointed out by Nilsson *et al.*[8] However, Matheson and Lee[9] reported a contradictory result, *i.e.*, that the main part of the interaction of $^1O_2$ with **1** is a chemical reaction.

Product Distribution. The dye-sensitized photooxidation of **1** is known to give (*N*-FK) and 3a-hydroxypyrrolidinoindole (HPI) *via* 3a-hydroperoxypyrro-



Scheme 3.

lidinoindole (HPPI)[4] (Scheme 3). The products of the reaction of chemically generated $^1O_2$ were compared with those for RB- and thionine-sensitized photooxidations. As already mentioned, the reaction mechanism is highly dependent on the type of sensitizer. The oxidation of **1** with RB proceeds exclusively by the $^1O_2$ mechanism, while with thionine it proceeds by a combination of $^1O_2$ and Type I mechanisms. Product ratio *N*-FK/HPI for the RB-sensitized photooxidation using a filter solution (aqueous $CaCl_2$–$CuCl_2$ solution, cutoff <550 nm) is the same as that obtained from the pure $^1O_2$ reaction (Table 4). However, when the photooxidation was carried out without filter, the yield of HPI decreased drastically. Savige[3] has reported that the photooxidation of **1** in water at 15—20 °C gives *N*-FK and HPI as the main product, while at temperatures above 20 °C it gives *N*-FK in improved yield but HPI in less yield, and that HPI is subjected to further photooxidation in the presence of MB. In fact, during the MB-sensitized photooxidation or direct irradiation (>290 nm) of HPI, at least 9 products were detected. Therefore, the low yield of HPI without filter solution may be due to its further photodecomposition by the action of shorter-wavelength light. Furthermore, the low yield of HPI in the thionine-sensitized photooxidation may be due to the co-occurrence of a free radical process (Type I mechanism). A similar result has been reported by Singh *et al.*,[23] who showed that product ratios *N*-FK/HPI in Sensitox II-sensitized photooxidation and in photophysically generated $^1O_2$ reaction are quite different from each other.

Although a number of interesting findings such as the formation of a tricyclic hydroperoxide like HPPI have been reported for the dye-sensitized photooxidation of indolic substrates including **1**,[1,4] the mechanism of the photooxidation has not yet be fully understood. The results described here demonstrate that the mechanisms of photooxidations of Trp, tryptamine, and their derivatives are strongly dependent on the type of sensitizers. The oxidation with RB sensitizer proceeds exclusively *via* the singlet oxygen process. However, if higher concentrations of substrates ($>10^{-3}$ M) were used, Type I process would become important. The oxidation with thionine sensitizer proceeds *via* a combination of Type I and II mechanisms even at lower concentrations of substrates. The rate constants of the reaction of $^1O_2$ with indolic substrates are somewhat smaller than those for the quenching reaction, indicating that the major part of the interaction of $^1O_2$ with Trp is the quenching process at least in aqueous methanol.

Table 4. Oxidation products of tryptophan under various conditions[a]

Reaction system	Experimental conditions	Product yield/%[b]	
		N-FK	HPI
EP	35 °C	23	42
Rose Bengal/O_2	>300 nm	30	1
	>550 nm[c]	23	44
Thionine/O_2	>300 nm	29	1
	>550 nm[c]	22	23

a) In 1:1 MeOH–H_2O. b) The yields were determined by HPLC analyses. c) Aqueous $CaCl_2$–$CuCl_2$ solution was used as a filter.

No variation in functional groups on side chains of Trp derivatives gives any significant effect on the rate of reaction with $^1O_2$ and on the extent of contribution of the $^1O_2$ mechanism under a given set of conditions. The product composition in the Rose Bengal-sensitized photooxidation of Trp under carefully controlled conditions is the same as that obtained in the reaction using chemically generated $^1O_2$. The present results suggest that the dye-sensitized photooxidation of tryptophan residues in a protein may porceed *via* a similar mechanism, and that the rate constant for the reaction of tryptophan residues with $^1O_2$ is of the same order of magnitude as that obtained for the present model compounds **1**—**5**.

References

1) J. D. Spikes and M. L. Macknight, *Ann. N.Y. Acad. Sci.*, **171**, 149 (1970); I. Saito, T. Matsuura, M. Nakagawa, and T. Hino, *Acc. Chem. Res.*, **10**, 346 (1977); M. Nakagawa, H. Watanabe, S. Kodato, H. Okajima, T. Hino, J. L. Flippen, and B. Witkop, *Proc. Natl. Acad. Sci., U.S.A.*, **74**, 4730 (1977); A. Fontana and C. Toniolo, *Prog. Chem. Org. Nat. Prod.*, **33**, 309 (1976) and references therein.

2) For example, R. S. Asquith and D. R. Rivett, *Biochim. Biophys. Acta*, **252**, 111 (1971); Z. Yoshida and M. Kato, *J. Am. Chem. Soc.*, **76**, 311 (1954).

3) W. E. Savige, *Int. Wool Text. Res. Conf., Vol. II*, **1975**, 570.

4) M. Nakagawa, S. Kato, S. Kataoka, and T. Hino, *J. Am. Chem. Soc.*, **101**, 3136 (1976).

5) I. Kralijic and I. Lindquist, *Photochem. Photobiol.*, **20**, 351 (1974).

6) R. S. Davidson and K. R. Trethewey, *J. Am. Chem. Soc.*, **98**, 4008 (1976).

7) K. Gollnick, *Adv. Photochem.*, **6**, 1 (1968).

8) R. Nilsson, P. B. Merkel, and D. R. Kearns, *Photochem. Photobiol.*, **16**, 117 (1972).

9) I. B. C. Matheson and J. Lee, *Photochem. Photobiol.*, **29**, 878 (1979).

10) G. J. Smith, *J. Chem. Soc., Faraday Trans. 2*, **74**, 1350 (1978).

11) C. Sconfienza, A. Van de Vorst, and G. Jori, *Photochem. Photobiol.*, **31**, 351 (1980).

12) E. Rossi, A. Van de Vorst, and G. Jori, *Photochem. Photobiol.*, **34**, 447 (1981).

13) I. Saito, T. Matsuura, and K. Inoue, *J. Am. Chem. Soc.*, **103**, 188 (1981).

14) C. S. Foote and T.-Y. Ching, *J. Am. Chem. Soc.*,

97, 6209 (1975).
15) N. Hasty, P. B. Merkel, P. Radlick, and D. R. Kearns, *Tetrahedron Lett.*, **1972**, 49.
16) R. Nilsson, P. B. Merkel, and D. R. Kearns, *Photochem. Photobiol.*, **16**, 109 (1972).
17) V. Kasche, *Photochem. Photobiol.*, **6**, 643 (1967).
18) Y. Usui and H. Koizumi, *Bull. Chem. Soc. Jpn.*, **40**, 440 (1967).
19) G. R. Fleming, A. W. E. Knight, J. M. Morris, R. J. S. Morrison, and G. W. Robinson, *J. Am. Chem. Soc.*, **99**, 4306 (1977).
20) P. R. Ogilby and C. S. Foote, *J. Am. Chem. Soc.*, **103**, 1219 (1981).
21) A. G. Kepka and L. I. Grossweiner, *Photochem. Photobiol.*, **16**, 305 (1972).
22) P. B. Merkel and D. R. Kearns, *J. Am. Chem. Soc.*, **94**, 7244 (1972).
23) A. Singh, M. J. Bell, G. W. Koroll, W. Kremers, and H. Singh, *Intl. Conf. on Oxygen and Oxy-radicals in Chem. and Biol., Austin*, 461 (1980).
24) B. A. Lindig and M. A. J. Rodgers, *Photochem. Photobiol.*, **33**, 627 (1981).