

cationic quenchers, MV^{2+} and Cu^{2+} , compared to K_{sv} 's in PVS solutions. The novel effects of PSS on the enhancement of the rates of electron transfer reactions due to longer excited lifetime and higher binding constant of a reacting species might lead to improvement of the efficiency of photoenergy conversion by proper choice of hydrophobic polyelectrolytes.

Acknowledgements. This work was supported by the Korea Science and Engineering Foundation. Y.H. Paik is a recipient of the Graduate Fellowship from KOSEF. We thank to KOSEF for the supports.

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- (20) In the absence of added salts, the addition of PVS enhanced K_{sv} by about 10^3 times,³ while we observed only 1-4 times enhancement of K_{sv} by PVS in 0.1 M NaCl solutions presumably due to masking of potential field of the polyanion by Na^+ .
- (21) The radius and length of monomer unit of PVS were estimated as 10 \AA and 2.5 \AA , respectively.* We assumed the length of phenyl group in PSS is 4 \AA . In our experimental condition of 0.1 M NaCl, PSS may not exist as extended rod-like polyanion due to reduced charge repulsion. Thus, volume fraction of the polyanion and binding constants of quenchers with PSS correspond to their upper limits.
- (22) There is good possibility that the quenchers, especially MV^{2+} , are associated with only one anionic site of polyelectrolytes. In this case, the binding constants of quenchers with PSS are reduced by half, and our argument on K 's is strengthened.

Methyl Linoleate Oxidation via Electron Transfer in Competition with 1O_2 Formation Photosensitized by N-Acetyl-L-Tryptophan and 3-Methyl Indole

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(Received June 7, 1985)

The efficiency of photosensitization of methyl linoleate (ML) oxidation by N-acetyl-L-tryptophan (NAT) and 3-methyl indole (scatole) was markedly enhanced by increased concentration of ML in ethanol solution. The fluorescence intensities of sensitizers were observed to be quenched by ML, indicating that ML interacts with the indole excited singlet state. The inhibition of photosensitization by azide demonstrated a possible role of singlet oxygen in the photosensitization. The steady state kinetic treatment of azide inhibition of photosensitization was expected to show linear increase of reciprocal yield of ML oxidation product vs. reciprocal ML concentration at constant azide concentration, but the actual slope was nonlinear. This indicates another competing reaction involved in the photosensitization. As a possible competing reaction, electron transfer from ML to the excited sensitizer was proposed, since the measured fluorescence quenching rate constant closely resembled electron transfer rate constant determined from ML concentration dependence of oxidation product formation.

Introduction

Irradiation of human skin with ultraviolet-B(UVB) light (290–320nm) induces delayed erythema (sunburn). Damage to cell membranes has been cited as a possible initiating photochemical process. The light-initiated disruption of cell membranes is believed to be caused by photooxidation of membrane lipids.^{1,2} The membrane lipids do not absorb light in the UVB range significantly. Therefore, photooxidation of lipid requires a chromophore absorbing UVB light as a photosensitizer. Kochevar³ proposed that tryptophan (Trp) in membrane protein is one of the most possible native chromophores functioning for this purpose. Supporting this idea, previous investigation⁴ has demonstrated that Trp in peptides photosensitizes the oxidation of methyl linoleate (ML) in ethanol solution. The photosensitization did not involve the photooxidation product of Trp, N-formylkynurenine which is a photodynamic sensitizer.^{5,6}

The mechanism of photosensitization by Trp has not been established yet. At first glance, the tryptophan in peptide appears to generate some singlet oxygen (1O_2) which reacts with ML to form a hydroperoxide, evidenced by observation that hydroperoxide formation is quenched by azide (N_3).⁴ Other results indicate that ML is oxidized by 1O_2 generated from methylene blue in ethanol solution.⁷ However, the azide inhibition of Trp-photosensitized oxidation of ML was not complete, but partial, indicating multiple competing reactions involved in the Trp-photosensitization. One of the competing reactions may be photoejection of an electron which subsequently forms superoxide ion (O_2^-) through interaction with molecular oxygen dissolved in solution. However, this pathway may not be important in ethanol solution, since the photoionization of Trp in alcohol is not observed at wavelengths longer than 270nm.⁸

Interestingly in the previous paper⁴, the relative photooxidation of ML sensitized by Trp was reported to depend on ML concentration. This was interpreted to be due to very efficient photosensitization by a small amount impurity in ML which was not detected even by HPLC and absorption spectroscopic method. However, this does not eliminate the possibility that photosensitization by Trp can be affected by ML. Thus, being interested in the nonsinglet oxygen mechanism of photosensitization by Trp, we reinvestigated the effect of ML concentration on Trp-sensitized photooxidation of ML in ethanol solution, using N-acetyl-L-tryptophan(NAT) and 3-methyl indole (scatole).

Experimental Section

Materials. N-acetyl-L-tryptophan(NAT) and 3-methyl indole(scatole) were used as supplied from research Plus Lab. and Sigma Chemical Co., respectively. Methyl linoleate(ML) obtained from Sigma Chemical Co. was purified by chromatography on silica gel, as described in the previous paper,⁴ and its purity was determined by observing a single spot on silica gel TLC(hexane/ether/acetic acid, 35/2/1 by volume) and by observing no absorption peak beyond 220nm which is characteristic of impurity.

Measurement of fluorescence quenching. Fluorescence spec-

tra of NAT and scatole were measured on Jovin Yvon JY-3 spectrofluorometer at ambient temperature. The quenching of NAT or scatole fluorescence was observed upon addition of ML, and its rate was determined from the Stern-Volmer equation (equation 1) by using least square analysis. Solutions of the fluorescers(0.03mM) and ML(0.01–0.05M) were deoxygenated by gentle bubbling with 99.99% nitrogen through the solutions in septum-capped quartz cuvette just before the measurement of fluorescence. Equation 1 is given by

$$\frac{F_o}{F} = 1 + k_q \tau_f (ML) \quad (1)$$

where F_o/F is the ratio of fluorescence intensities of NAT or scatole in the absence or presence of ML, and k_q and τ_f are quenching rate constant and fluorescence lifetime, respectively.

Irradiation. Irradiation was performed in a modified Merry-Go-Round⁹ attached with a Bausch & Lomb 200W medium pressure mercury lamp. 1 ml samples were irradiated for 90 min in matched pyrex test tubes which transmit less than 10% at 270nm and lower wavelength. The incident radiation was filtered with 1cm of water. Irradiation of NAT was also performed in parallel with all samples to correct day-to-day variation in fluence rate.

Gas chromatography. Irradiated samples were analyzed by Yanaco G180 gas chromatography with flame ionization detector immediately after irradiation. The column (3.0mm × 1.5m) was packed with 20% DEGS on Chromosorb W and maintained at 170°C.

Results and Discussion

Photoreaction of ML was performed in the presence and absence of 0.3mM scatole or NAT. The amount of photoreacted ML was determined by monitoring the decrease of ML peak

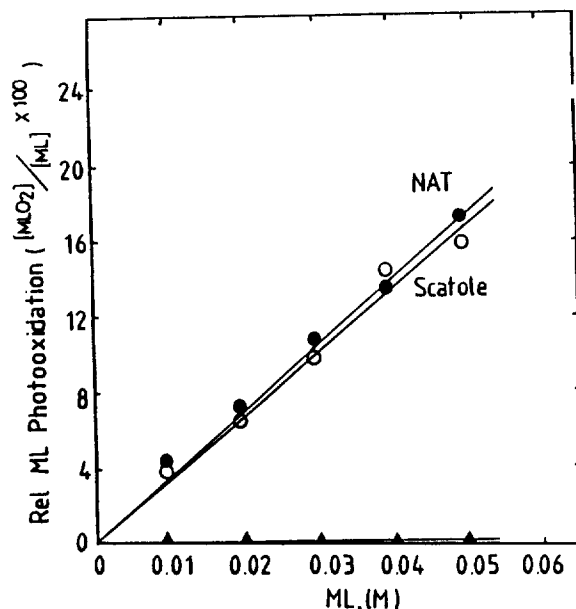


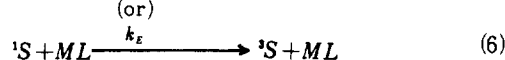
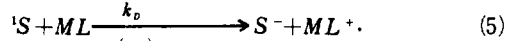
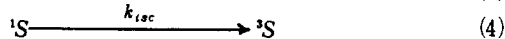
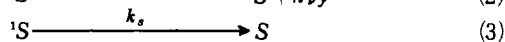
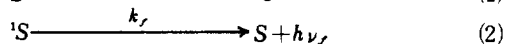
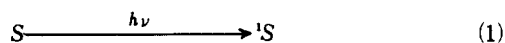
Figure 1. The effect of ML concentration on its photooxidation in the presence and absence of scatole and NAT. ML in air saturated ethanol was irradiated with $\lambda > 270$ nm for 90 min along (▲) or with 0.3mM NAT (●) and scatole (○). The relative decrease in area of GC peak attributed to ML photooxidation is plotted vs. ML concentration. Points represent the mean value from duplicate results.

area of gas chromatography. The photoreaction of ML is assigned to be photooxidation, since no change of ML peak was observed when irradiation was done with N_2 -purged sample.

Figure 1 shows the relative photooxidation of ML as a function of ML concentration in the presence of scatole or NAT. This result is in good agreement with that monitored by HPLC as reported previously.⁴ Negligible oxidation occurred when ML was irradiated alone. These results indicate that NAT or scatole photosensitizes oxidation of ML, and that the relative photosensitization is increased with ML concentration. One would not expect the change in relative photosensitization with ML concentration, if the photosensitizing ability of sensitizers are not affected by pure ML.

In order to explore the effect of ML on the photosensitizing ability of the sensitizers, primarily we have measured fluorescence spectra of the sensitizers in the presence and absence of ML. The fluorescence emission maxima consistently appeared at 347nm with excitation wavelength 285nm or 295nm, regardless whether ML present or not (data not shown). This indicates that microenvironment of sensitizers is not changed. However, fluorescence intensities of sensitizers were observed to be quenched by ML. Presented in Figure 2 are the results, plotted in Stern-Volmer fashion (see equation I), of ML quenching studies of scatole and NAT in ethanol. Assuming 5.2 and 6.4 nsec as the fluorescence lifetimes of scatole and NAT¹⁰ respectively, the bimolecular collisional quenching rate constant is approximately $0.5-0.6 \times 10^{10} M^{-1} s^{-1}$. This value indicates that the diffusion-controlled reaction is effective in quenching of fluorescence of scatole or NAT.

Some olefins structurally similar to ML quench the fluorescence of aromatic fluorophores by forming a transient exciplexes which are often non-fluorescent^{11, 12} and promoting electron transfer¹³ or intersystem crossing.¹⁴ Thus, it can be speculated that the following photophysical processes of sensitizers(S) such as NAT or scatole are possible in the presence of ML under anaerobic condition (Scheme I),



where k_f 's represent rate constants for fluorescence emission (k_f), internal conversion (k_a), intersystem crossing (k_{isc}), ML-enhanced electron transfer (k_e) and ML-enhanced intersystem crossing (k_i).

Figure 3 shows that the relative photooxidation of ML ($[MLO_2]/[ML]_0$) sensitized by NAT and scatole is inhibited as the concentration of azide ion, a singlet oxygen quencher, increase, supporting the possible role of singlet oxygen in the oxidation of ML again as previously observed⁴. If step (6) in scheme I is dominant for the fluorescence quenching, compared to step (5), the formation and dissipation of singlet oxygen can be processed competitively in the presence of sensitizer, ML and a singlet oxygen quencher(Q), as shown in the following Scheme II,

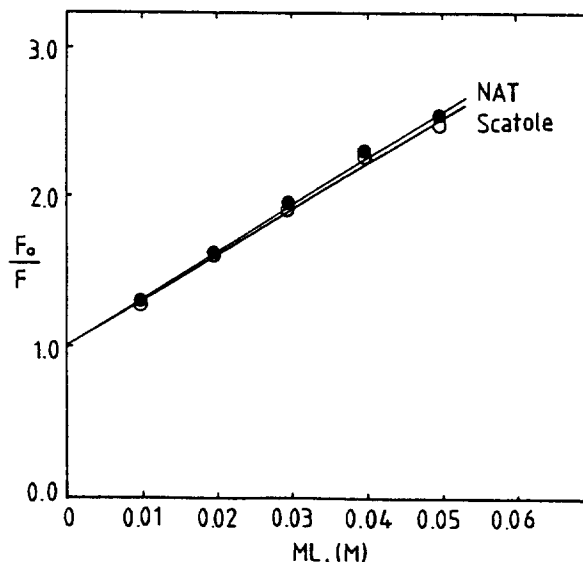
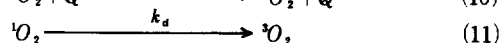
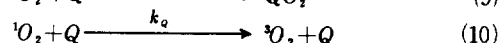
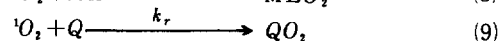
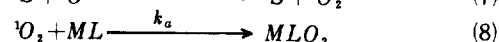
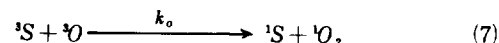


Figure 2. Stern-Volmer plot for ML-induced fluorescence quenching of NAT(●) and scatole(O) in deaerated ethanol. The concentration of each fluorophore was 0.03mM. $\lambda_{em}^{max} = 347nm$ $\lambda_{ex} = 285nm$ or 295nm. Slit width for excitation = 10nm.



where k 's are rate constants for energy transfer from 3S to 3O_2 (k_o), 1O_2 -induced oxidation of ML (k_a) chemical quenching of 1O_2 (k_r), physical quenching of 1O_2 (k_q) and autoddecay of 1O_2 (k_d). Quenching of singlet sensitizer is negligible, since the short lifetime of the singlet state requires a very high concentration of azide ion. The steady-state kinetic treatment of Scheme I and Scheme II gives the following equation II for the quantum yield of formation of product MLO_2 (ϕ_{MLO_2}).¹⁵

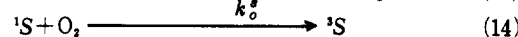
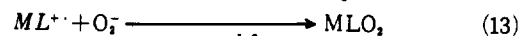
$$\phi_{MLO_2} = \left(\frac{k_{isc}}{k_s + k_r + k_{isc}} \right) \left(\frac{h_a [ML]}{k_a [ML] + (k_r + k_q) [Q] + k_d} \right) \quad (II)$$

This equation II can be inverted and reduced to the following equation III.

$$\phi_{MLO_2}^{-1} = \tau_{isc}^{-1} \left(1 + \frac{k_d + (k_r + k_q) [Q]}{k_a} [ML]^{-1} \right) \quad (III)$$

Equation III allows linear plots of $[MLO_2]^{-1}$ (the amount of product formed in a given time) vs $[ML]^{-1}$, showing straight lines with common intercept. However, the actual slope increased as the $[ML]$ increased in non-linear fashion for both NAT and scatole photosensitized ML oxidation (see Figure 4). This indicates that another competing reaction is involved in the photosensitization of NAT or scatole for ML oxidation.

Alternatively, electron transfer (step (5)) may be dominant for quenching of NAT or scatole fluorescence. Thus, the following kinetic scheme III may be considered for the formation of a photooxidation product of ML as a result of the sensitized electron transfer between sensitizer and ML.



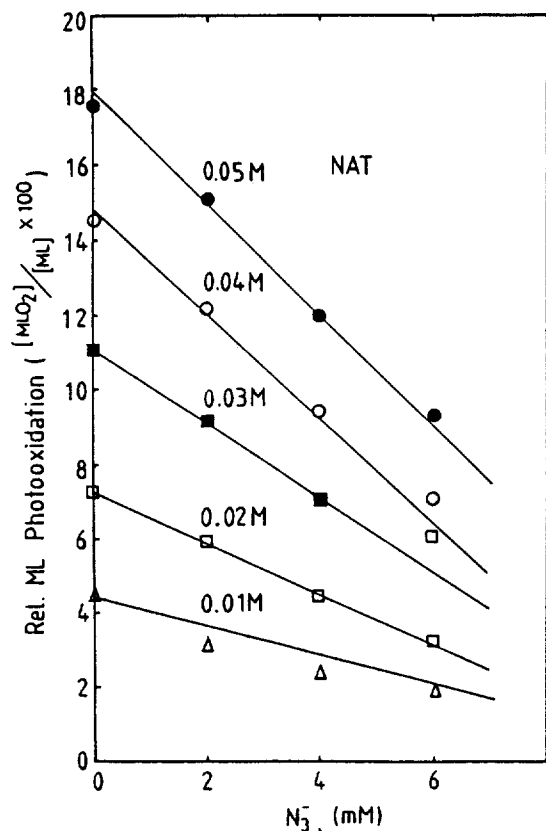


Figure 3. The effect of azide ion concentration on the relative photooxidation of ML in the presence of NAT with respect to ML concentration, 0.01M(Δ), 0.02M(\square), 0.03M(\blacksquare), 0.04M(\circ) and 0.05M(\bullet).

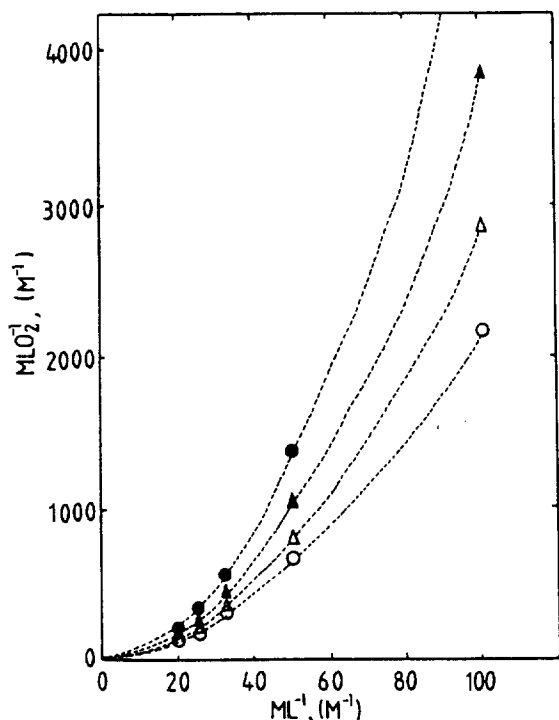


Figure 4. Reciprocal plot of ML oxidation product yield vs. reciprocal ML concentration with respect to various concentrations of azide ion; 0mM(\circ), 2mM(Δ), 4mM(\blacktriangle) and 6mM(\bullet). See detail in text.

The quantum yield for formation of MLO_2 (ϕ_{MLO_2}) can be obtained by steady-state treatment of kinetic Schemes I and III, assuming F as the fraction of the radical ion pair ($S^- + ML^+$) which forms MLO_2 (see equation IV).¹⁶

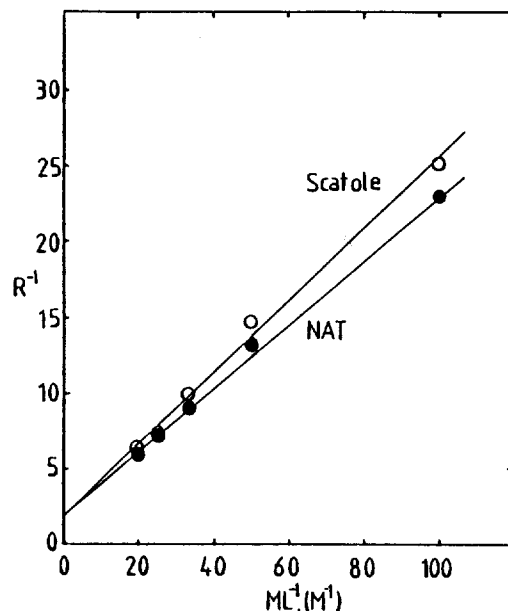


Figure 5. Reciprocal plot of relative quantum yield for product formation vs. reciprocal ML concentration. Data were taken from Figure 1. (\bullet), for NAT, (\circ), for scatole.

TABLE 1: Rate Constants for Fluorescence Quenching Electron Transfer of Sensitizers for Methyl Linoleate

Sensitizer	$10^{10}k_D^a$ $M^{-1}s^{-1}$	$10^{10}k_q^b$ $M^{-1}s^{-1}$
NAT	0.20	0.61
scatole	0.16	0.47

^aRate constant for electron-transfer determined from ML concentration dependence of oxidation product formation. ^bRate constant for fluorescence quenching from Stern-Volmer plot (Figure 2)

$$\phi_{MLO_2} = F \left(\frac{k_D [ML]}{k_f + k_s + k_{isc} + k_o^s [O_2] + k_D [ML]} \right) \quad (IV)$$

The reciprocal value of relative quantum yield for product formation (R) is proportional to ϕ_{MLO_2} , giving equation V,

$$1/R = F' \left(1 + \frac{k_f + k_s + k_{isc} + k_o^s [O_2]}{k_D} \right) \frac{1}{[ML]} \quad (V)$$

where F' is the proportionality including F . Figure 5 shows the plot of R^{-1} vs $[ML]^{-1}$, giving linearity as expected. The k_D values were determined from the slopes and intercepts by assuming 5.2 and 6.4 nsec as fluorescence life-times of NAT and scatole, respectively.¹⁰ The rate constant for fluorescence quenching (k_o^s) of NAT or scatole by oxygen was measured to be $1.2 \times 10^{10} M^{-1} s^{-1}$. Table 1 lists the values of k_D which are almost identical with k_f within experimental error. This suggests the possibility of involvement of electron-transfer mechanism in NAT or scatole photosensitization of ML oxidation.

In conclusion, tryptophan seems to sensitize photooxidation of methyl linoleate through electron-transfer in competition with 1O_2 formation. The electron transfer may be more effective for photosensitization of oxidation of ML at higher concentration, because the fluorescence quenching of sensitizer by ML is involved in the first step of oxidation.

Acknowledgement. The authors wish to express gratitude to the Korea Science and Engineering Foundation for financial support of this work.

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A Stochastic Investigation of a Dynamical System Exhibiting the Second-Order Phase Transition

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An approximate solution of the Fokker-Planck equation with the nonlinear drift term due to a Schlögl model is obtained and the result is compared with the methods proposed by Suzuki. Also the effect of nonlinearity on the correlation length at the stable steady state is studied.

Introduction

In recent years, there have been considerable interests in the behavior of systems far from the thermodynamic equilibrium, which exhibits instability. Such phenomena are observed in many fields¹⁻⁴. Especially, the laser model⁵ draws a great attention. Recent investigations of several authors^{6, 7} have provided the analogy between transitions in unstable systems and phase transitions. Their theory is applicable to any system whose macroscopic behavior is governed by nonlinear evolution equations.

If there is no random force which is caused by internal microscopic fluctuations, a system in an unstable state does not undergo decaying process. Once the decay of the unstable state is initiated, fluctuations are amplified by the linear contribution which shows exponential divergence. Later, these fluctuations are affected by the nonlinear effect and then have the

finite steady state value. This fact has been studied from the Langevin eq. by Suzuki⁸ with his scaling theory and also by Valsakumar and his coworkers.⁹

Also several authors have tried to obtain the probability distribution function satisfying the Fokker-Planck equation. Among them, Suzuki^{8, 10} has divided the whole range of time into three parts, that is, initial, intermediate and final regimes and introduced the scaling theory connecting the initial and intermediate regimes. This theory is successful in describing the intermediate regime but does not describe the final steady state properly. Later, he has proposed the unified theory¹¹ using the properties of the exponential streaming operator. In addition, Valsakumar¹² has obtained the formally exact probability distribution function using Trotter's formula, but it has a rather complicated form and is difficult to handle.

In this work we study a chemical system which can be described by a stochastic variable governed by a Fokker-Planck equation. The nonlinear drift term in the Fokker-Planck equation

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