Kinetics of Photoperoxidation in Solution

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To the extent that life on earth is sustained by the sun's energy, transmitted through space as electromagnetic radiation, the interaction of this radiation with the biosphere is a matter of vital concern to humanity. In turn, the interpretation of photobiological phenomena at the molecular level relies heavily on the outcome of photochemical investigations of simple model systems. Thus visual response is triggered by photoisomerization of the pigment retinal,[1] an example of the extensively studied photochemical cis–trans isomerization of olefins,[2] while the photoaddition of unsaturated molecules to produce cyclobutane derivatives[3] has served as a model for the intrastrand photodimerization of adjacent thymine residues associated with the photoinactivation of deoxyribonucleic acid (DNA).[4]

Carbohydrates, proteins, nucleic acids, and other components of biological systems do not usually absorb solar radiation in the spectral region >300 nm transmitted by the ozone layer in the upper atmosphere. Consequently the initiation of photobiological processes often depends on light absorption by accessory pigments or sensitizers of which chlorophyll and the carotenoids are examples. A physiologically important class of sensitized photobiological reactions is that in which the damage or destruction of systems, ranging from enzymes and nucleic acids, through viruses and cells to plants, animals, and man, is accompanied by the consumption of molecular oxygen.[5] The characteristics of this so-called photodynamic action are remarkably similar to those exhibited by the photoperoxidation of unsaturated molecules, the detailed kinetic sequence of which is moderately well resolved. It is therefore of interest to examine the extent to which this reaction can serve as a model for photodynamic action.

The Photoperoxidation Reaction

The sensitized addition of molecular oxygen to an unsaturated organic substrate M is represented by the overall process

$$M \xrightarrow[O_2]{S,\ h\nu} MO_2$$

in which the light-absorbing sensitizer S remains chemically unchanged (except in the case of autoperoxidation described below) and the peroxide or hydroperoxide MO_2 is usually the sole product. The term photoperoxidation was introduced[6] to distinguish this reaction from such photooxidative processes as electron or H-atom transfer.

Examples[7] of reactive substrates or oxygen acceptors include compounds with an isolated carbon–carbon double bond and an allylic hydrogen atom which form unsaturated hydroperoxides, cyclic conjugated dienes which form annular peroxides, a process exploited commercially[8] for the production of ascaridole from α-terpinene using chlorophyll as sensitizer in the presence of sunlight, and certain catacondensed aromatic hydrocarbons and their derivatives, which are capable of acting as their own sensitizers (S ≡ M) in a process of autoperoxidation.

Product analysis,[9] and the appearance of isosbestic points in the absorption spectra of aromatic hydrocarbons undergoing autoperoxidation,[6] define the overall reaction stoichiometry, and the reaction rate has been measured as the rate of substrate depletion,[6] of oxygen consumption,[10] or of product accumulation.[11] For a given system the rate varies directly as the absorbed light intensity I_a[6,11] and the overall quantum yield $\gamma(MO_2)$ increases wih both the concentration of dissolved oxygen, $[O_2]$, and substrate concentration, [M], to limiting values;[6,11,12] the reaction is not inhibited by products provided these do not absorb the actinic radiation.[6] The simplest reaction sequence qualitatively consistent with these observations is that of Scheme I. This provides

Scheme I

$$S + h\nu \longrightarrow S^*$$

$$S^* \longrightarrow S\ (+h\nu) \qquad (a)$$

$$S^* + O_2 \longrightarrow X \qquad (b)$$

$$X + M \longrightarrow MO_2 + S \qquad (c)$$

$$X \longrightarrow S + O_2 \qquad (d)$$

expression I for the overall quantum yield in terms of

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$$\gamma(MO_2) = \frac{-1}{I_a}\frac{d[M]}{dt} = \frac{1}{I_a}\frac{d[MO_2]}{dt} = \gamma_{S^*}\left\{\frac{k_b[O_2]}{k_a + k_b[O_2]}\right\}\left\{\frac{k_c[M]}{k_d + k_c[M]}\right\} \quad (I)$$

the rate constants k_i of the ith process, where γ_{S^*} is the quantum yield of formation of the unspecified excited state S* of the sensitizer, and the substrate reactivity parameter[13] $\beta = k_d/k_c$ is available from measurements of $\gamma(MO_2)$ (or relative yield) as a function of substrate concentration [M].

Foote[13] has discussed the evidence supporting the role of the low-lying singlet state of molecular oxygen, $O_2(^1\Delta_g)$ (at ~8000 cm^{-1} above the $O_2(^3\Sigma)$ ground state), as the reaction intermediate X in solution, and its physical and chemical properties have been extensively reviewed.[14] The analysis of kinetic data presented below is based on the assumption of an $O_2(^1\Delta)$ reaction intermediate, although it is recognized that this is kinetically indistinguishable from a sensitizer–oxygen complex (of sensitizer-independent reactivity) and that to date no spectroscopic evidence has been reported for the presence of $O_2(^1\Delta)$ in a condensed system undergoing photoperoxidation.

Knowing the identity of the "reactant," or electronically excited species responsible for its initiation, is essential to the complete understanding of any photochemical reaction. In the present context this concerns the role of excited singlet and triplet states of the sensitizer, either or both of which may generate $O_2(^1\Delta)$ in the spin-allowed exothermic oxygen-quenching processes 1 (if the sensitizer singlet–

$$^1S^* + O_2(^3\Sigma) \longrightarrow {}^3S^* + O_2(^1\Delta) \quad (1)$$

triplet splitting $\Delta E_{ST} > 8000$ cm^{-1}) and 2 (if the

$$^3S^* + O_2(^3\Sigma) \longrightarrow S + O_2(^1\Delta) \quad (2)$$

sensitizer triplet state energy $E_T > 8000$ cm^{-1}). The $O_2(^1\Delta)$ precursor is identified kinetically by an analysis of the dependence of the overall quantum yield on the concentrations both of dissolved oxygen and of the inhibitor azulene.

Oxygen Dependence of Quantum Yield at High Oxygen Concentrations. Equation I may be written in the form

$$\gamma(MO_2) = \gamma_{S^*}\left\{\frac{k_b\tau_{S^*}[O_2]}{1 + k_b\tau_{S^*}[O_2]}\right\}\left\{\frac{[M]}{\beta + [M]}\right\}$$

where τ_{S^*} ($=k_a^{-1}$) is the lifetime of the $O_2(^1\Delta)$ precursor S* in the absence of oxygen. Accordingly if γ_{S^*} is insensitive to oxygen concentration, $\gamma(MO_2)$ will also be independent of this variable when this substantially exceeds $(k_b\tau_{S^*})^{-1}$. Gollnick and Schenck[7] find that this condition is satisfied when $[O_2] > 2 \times 10^{-3}$ M for the photoperoxidation of 2,5-dimethylfuran sensitized by a series of halogenated fluorescein dyes, and conclude that, since $\tau_{S^*} \gg 1/(2 \times 10^{-3}k_b) \sim 50$ nsec, which exceeds the fluorescence lifetime, the triplet state of these dyes is the precursor of $O_2(^1\Delta)$ (process 2); this is to be expected for these sensitizers in which $\Delta E_{ST} < 8000$ cm^{-1}, and process 1 is energetically prohibitive.

Stevens and Algar[6,15] have presented a detailed analysis of the oxygen concentration dependence of quantum yields of photoperoxidation sensitized by a series of aromatic hydrocarbons in which ΔE_{ST} exceeds the excitation energy of $O_2(^1\Delta)$ (8000 cm^{-1}). In addition to the possible formation of $O_2(^1\Delta)$ from sensitizer singlet and/or triplet states these authors also consider triplet-state formation from oxygen quenching of the sensitizer singlet state in addition to the unimolecular intersystem crossing route. It is convenient to define the following parameters: $P(O_2)$, the probability that a sensitizer singlet state is quenched by oxygen at the prevailing concentration, independently available from oxygen quenching of sensitizer fluorescence as $(F_0 - F)/F_0$, where F_0 is the fluorescence intensity in the absence of oxygen;[16] α, the probability that oxygen quenching of the singlet state generates $O_2(^1\Delta)$; δ, the probability that oxygen quenching of the singlet state produces the sensitizer triplet state; ϵ, the probability that oxygen quenching of the triplet state produces $O_2(^1\Delta)$.

The overall quantum yield, γ_Δ, of $O_2(^1\Delta)$ formation, available experimentally as $\gamma(MO_2)(1 + \beta/[M])$, is given by eq II if oxygen quenching of the

$$\gamma_\Delta = \alpha P(O_2) + \gamma_{3S^*}\varepsilon \quad (II)$$

sensitizer triplet state may be assumed complete under conditions where $P(O_2)$ is significant. Since the quantum yield γ_{3S} of triplet-state formation may be expressed as the sum of the intersystem crossing yield $\gamma_{IS}(1 - P(O_2))$ in the presence of oxygen and the oxygen-induced singlet–triplet conversion efficiency $\delta P(O_2)$, eq II reduces to eq III, which describes the

$$\gamma_\Delta = \varepsilon\gamma_{IS} + P(O_2)[\alpha + \varepsilon(\delta - \gamma_{IS})] \quad (III)$$

linear dependence of γ_Δ on $P(O_2)$, measured independently as $1 - F/F_0$, exhibited by the data shown in Figure 1 for a number of sensitizers.

Within the limits of experimental error the intercepts $\gamma_\Delta = \epsilon\gamma_{IS}$ at $P(O_2) = 0$ of the data lines in Figure 1 are equal to published values of γ_{IS}(naphthacene)[17] or of $1 - \gamma_F$ for the sensitizers examined, indicating a value of unity for ϵ; it is there concluded that the energy transfer process 2 is predominantly responsible for oxygen quenching of the sensitizer triplet state either directly or, in the case of higher energy triplet states ($E_T > 13000$ cm^{-1}), *via* the intermediate production[18] of $O_2(^1\Sigma)$ (at 13000 cm^{-1}) which must undergo a rapid quantitative relaxation to $O_2(^1\Delta)$.[19]

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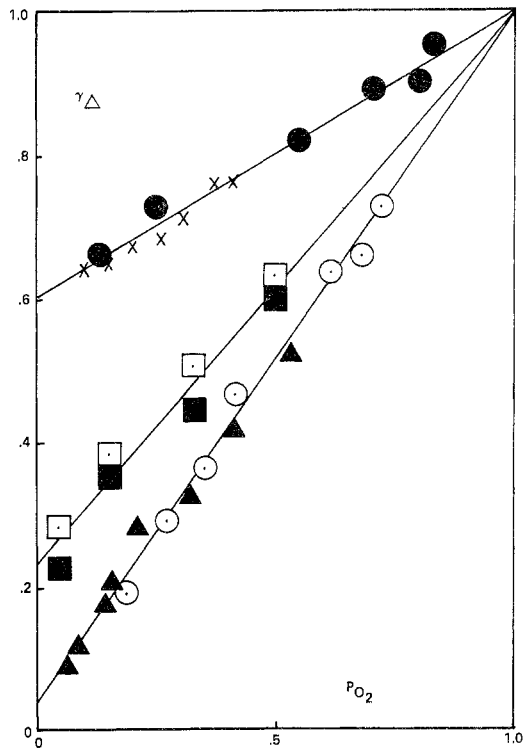

Figure 1. Plot of $O_2(^1\Delta)$ yield γ_Δ against fraction $P(O_2)$ of sensitizer singlet states quenched by O_2 (eq III). (●) 9,10-Dimethyl-1,2-benzanthracene (DMBA); (×) naphthacene; (□) anthanthrene with DMA acceptor; (■) anthanthrene with DMBA acceptor; (▲) rubrene; (○) 9,10-dimethylanthracene (DMA). Solvent benzene at 25°.

The data lines in Figure 1 extrapolate to a limiting quantum yield of $O_2(^1\Delta)$ formation of unity when oxygen quenching of the sensitizer singlet state is complete ($P(O_2) = 1$), or in terms of eq III (with $\epsilon = 1$)

$$\alpha + \delta = 1$$

This condition eliminates process 1 (which with process 2 would lead to a limiting value of 2 for γ) and is most simply interpreted in terms of process 3 ($\delta =$

$$^1S^* + O_2(^3\Sigma) \longrightarrow {}^3S^* + O_2(^3\Sigma) \qquad (3)$$

1, $\alpha = 0$), or the formally spin-forbidden process 4 (δ

$$^1S^* + O_2(^3\Sigma) \longrightarrow {}^1S + O_2(^1\Delta) \qquad (4)$$

$= 0$, $\alpha = 1$). This kinetic ambiguity is further resolved by an examination of the effects of the energy-acceptor azulene on the overall quantum yield.

Azulene-Inhibited Reaction. Azulene A may intercept both the singlet and triplet states of 9,10-dimethylanthracene (DMA) in the energy-transfer processes 5 and 6, to produce the lowest excited sin-

$$^1DMA^* + A \longrightarrow DMA + {}^1A^* \qquad (5)$$

$$^3DMA^* + A \longrightarrow DMA + {}^3A^* \qquad (6)$$

glet and triplet states of azulene which undergo rapid nonradiative relaxation[20] and are not subject to oxygen quenching in air-saturated benzene. Since DMA itself has a negligible intersystem crossing yield (Figure 1), the addition of azulene should reduce the quantum yield of DMA autoperoxidation according to relationship IV ($\alpha + \delta = 1$), where the

$$\frac{\gamma(MO_2)}{\gamma(MO_2)^A} = \frac{F}{F_A}\left\{1 + \frac{\delta k_6[A]}{k_2[O_2] + \alpha k_6[A]}\right\} \qquad (IV)$$

quotient F/F_A describing azulene quenching of the singlet state (process 5) is available from independent measurements of azulene quenching of DMA fluorescence, F, and the term in parentheses arises from the triplet-state quenching process 6. The experimental quantity $\gamma(MO_2)F_A/\gamma(MO_2)^AF$ is found[21] to be a linear function of azulene concentration, indicating azulene interception of the DMA triplet state with $\alpha \ll \delta > 0$; in the absence of intersystem crossing, this triplet state must originate from oxygen quenching of the DMA singlet state (process 3). This evidence in support of process 3 as that predominantly (if not wholly) responsible for oxygen quenching of the sensitizer singlet state is consistent with the expectation that $k_3 \gg k_4$ on the grounds of spin conservation, and is confirmed by the recent pulsed-laser studies of Potashnik, Goldschmidt, and Ottolenghi[22] who find that $\delta = 1.0 \pm 0.2$ for a series of eight aromatic hydrocarbons in toluene.

A quantitative analysis of the data in terms of eq IV provides the value $k_2 = 3.3 \pm 0.5 \times 10^9\ M^{-1}\ sec^{-1}$, which is lower by a factor of 9 than the diffusion-limited rate constant $k_3 = 3.15 \pm 0.20 \times 10^{10}\ M^{-1}\ sec^{-1}$ for oxygen quenching of the corresponding singlet state; this is consistent with a spin statistical factor of $\frac{1}{9}$ anticipated for the detailed quenching sequence[23]

$$^3S^* + O_2(^3\Sigma) \longrightarrow \begin{cases} {}^5(S\text{-}O_2) \longrightarrow {}^3S^* + O_2(^3\Sigma) \\ {}^3(S\text{-}O_2) \longrightarrow {}^3S^* + O_2(^3\Sigma) \\ {}^1(S\text{-}O_2) \longrightarrow {}^1S + O_2(^1\Delta) \end{cases}$$

in which only one in nine of the sensitizer–oxygen complexes ($S\text{-}O_2$) has the resultant spin angular momentum of the products $^1S + O_2(^1\Delta)$. Direct measurements of k_2 by Patterson, Porter, and Topp[24] have shown that the encounter quenching probability increases with decreasing energy of the triplet state to a limiting value of $\frac{1}{9}$ for the anthracene triplet state at 14,700 cm^{-1}.

Oxygen Dependence of Quantum Yield at Low Oxygen Concentrations. At sufficiently low concentrations of dissolved oxygen unimolecular relaxation of the sensitizer triplet state of lifetime $^3\tau_S$ will effectively compete with the oxygen quenching process 2. This introduces an additional term to the quantum yield expression which may now be written as

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$$\gamma(MO_2) = \left\{\frac{\gamma_{IS} + k_3{}^1\tau_S[O_2]}{1 + k_3{}^1\tau_S[O_2]}\right\}\left\{\frac{k_2{}^3\tau_S[O_2]}{1 + k_2{}^3\tau_S[O_2]}\right\}\left\{\frac{[M]}{\beta + [M]}\right\} \quad (V)$$

where ${}^1\tau_S$ denotes the lifetime of the sensitizer singlet state. Since ${}^1\tau_S$ is orders of magnitude shorter than ${}^3\tau_S$ the overall yield is limited by oxygen quenching of the singlet state (described by the first term in equation V) at all oxygen concentrations for those sensitizers with high fluorescence yield ($\gamma_{IS} \simeq 0$), but is limited by oxygen quenching of the triplet state (defined by the second term in V) when γ_{IS} is significant.

Dependence of Quantum Yield on Substrate Concentration. In accordance with eq V the slope/intercept ratio of the observed linear function $\gamma(MO_2)^{-1}$ ($[M]^{-1}$) at constant oxygen concentration provides the substrate reactivity parameter $\beta = k_d/k_c$ which varies with substrate over five orders of magnitude[13] or from 55 M for (relatively unreactive) cyclohexene to 3×10^{-4} M for (reactive) DMA and rubrene. The independence of β on the sensitizer, and its identity with the reactivity parameter for the same substrate toward addition of chemically generated $O_2(^1\Delta)$, provide the most compelling evidence for the intermediary role of $O_2(^1\Delta)$ in the photosensitized reaction.[13] In this case k_d describes the unimolecular relaxation of $O_2(^1\Delta)$ (process 8) of lifetime $\tau_\Delta = 1/k_8$, and the marked dependence of β on substrate must originate in a similar variation of the rate constant k_c describing substrate–singlet oxygen addition (process 7). Koch[25] has investigated the temperature dependence of β for 46 substrates sensitized by ten different dyes in a series of pure and mixed hydroxylic solvents over a subambient temperature range of up to 140°. As shown schematically in Figure 2, β values for these substrates exhibit four types of temperature dependence in this range depending on the magnitude of β at 20°; this in turn is determined by the relative magnitudes of the rate constants describing dissociation (k_R) and peroxide formation (k_H) of the substrate–oxygen complex ${}^1(M\text{–}O_2)$ formed at the diffusion-limited rate $k_D[M][O_2(^1\Delta)]$ in the detailed reaction sequence

$$O_2(^1\Delta) + M \underset{k_R}{\overset{k_D}{\rightleftharpoons}} {}^1(M\text{–}O_2) \xrightarrow{k_H} MO_2$$

in terms of which

$$\beta = k_8/k_7 = k_8(k_R + k_H)/k_D k_H \quad (VI)$$

β therefore ranges from a diffusion-limited value ($k_H \gg k_R$) of $k_8/k_D < 0.002$ M for very reactive substrates to reaction-limited values ($k_H \ll k_R$) of $k_8 k_R/k_D k_H > 0.05$ M for relatively inert acceptors at ambient temperature, and the respective temperature dependence illustrated by curves I and IV of Figure 2 provide the activation energies

$$E_\beta{}^I = E_D - E_8$$

$$E_\beta{}^{IV} = E_D + E_H - E_R - E_8$$

The close correspondence of $E_\beta{}^I$ with the activation energy for viscous flow E_D for most solvents examined indicates that $\tau_\Delta = 1/k_8$ is essentially independent of temperature over the range examined, while the argument that $E_R \sim E_D$ for a weakly bound complex leads to the conclusion that $E_\beta{}^{IV} \sim E_H$ which varies from 2.3 kcal/mol for 2-methyl-4-phenylbutene ($\beta = 0.15$ M) to 10 kcal/mol for 2-butene ($\beta = 12.5$ M).

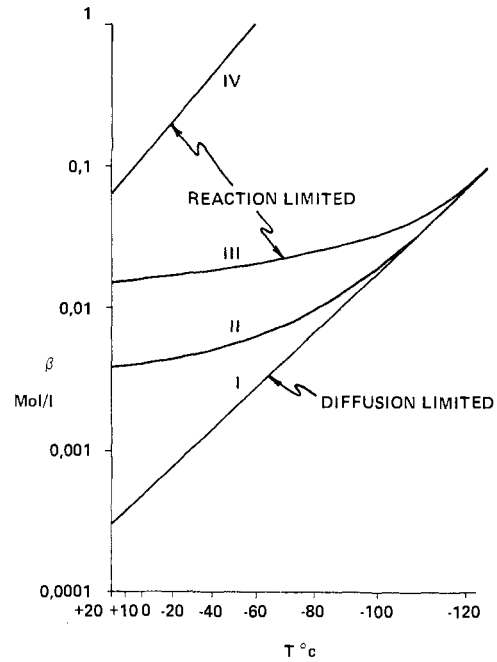

Figure 2. Schematic variation of reactivity parameter β with temperature (after Koch).[25]

Curves II and III of Figure 2 describe a transition from reaction-limited to diffusion-limited addition of $O_2(^1\Delta)$ to substrates of intermediate reactivity at ambient temperature as the temperature changes.

Relaxation and Quenching of $O_2(^1\Delta)$. The radiative lifetime of $O_2(^1\Delta)$ estimated from the integrated absorption coefficient is 2700 sec or 45 min.[26] In air at atmospheric pressure this is reduced to 0.088 sec due largely to the quenching process

$$O_2(^1\Delta) + O_2(^3\Sigma) \longrightarrow 2O_2(^3\Sigma) \quad (9)$$

for which a rate constant of 1.3×10^3 M^{-1} sec^{-1} has been reported.[27]

Foote and coworkers[28] have shown that the remarkably efficient inhibition of photosensitized peroxidation by β-carotene in solution is accompanied by cis–trans isomerization of this inhibitor which, however, is not otherwise significantly consumed. These observations are consistent with the energy transfer process 10 which provides an upper limit of

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$$O_2(^1\Delta) + \beta\text{-car} \longrightarrow O_2(^3\Sigma) + {}^3\beta\text{-car} \quad (10)$$

22.5 kcal/mol on the triplet-state energy of β-carotene, together with a logical interpretation of the protective action of carotenoids in photosynthetic systems against the damaging effects resulting from chlorophyll sensitization.[29] Quantitative studies[28] of the β-carotene inhibition of Methylene-Blue sensitized peroxidation of 2-methyl-2-pentene in benzene–methanol solutions lead to a value of $k_8/k_{10} = 3 \times 10^{-6}$ M, indicating that the transfer process 10 is some two orders of magnitude more efficient than $O_2(^1\Delta)$ addition to the most reactive acceptor ($\beta = 3 \times 10^{-4}$ M for DMA). If k_{10} is assigned an upper limit equal to the diffusion-controlled value of 3×10^{10} M^{-1} sec^{-1} for the oxygen quenching of singlet states in benzene, a lower limit of the lifetime τ_Δ of $O_2(^1\Delta)$ in the same solvent may be computed as

$$\tau_\Delta = 1/k_8 \geq k_{10}/(k_8 \times 3 \times 10^{10}) \sim 11\ \mu\text{sec}$$

Recently Merkel and Kearns[30] have measured the $O_2(^1\Delta)$ decay constant k_8 indirectly by monitoring the depletion rate of a reaction substate M following laser excitation of the sensitizer. For small changes in substrate concentration the relaxation equation

$$-d[M]/dt = k_7[O_2(^1\Delta)][M] = k_7[O_2(^1\Delta)]_0[M]\ \exp[-(k_8 + k_7[M])t]$$

is integrated to yield

$$[M] - [M]_\infty \simeq \left[\frac{k_7[O_2(^1\Delta)]_0[M]}{k_8 + k_7[M]}\right] \exp[-(k_8 + k_7[M])t]$$

$$-\frac{d\ \log\ OD}{dt} \simeq (k_8 + k_7[M]) \times 2.3$$

where [M] is the final substrate concentration and $\Delta D = \epsilon d([M] - [M]_\infty)$ denotes the change in optical density of substrate. Measurements of the relaxation time $(k_8 + k_7[M])^{-1}$ at two concentrations of substrate are sufficient to obtain k_8 (and k_7) which exhibits the hitherto unsuspected dependence on solvent shown in Table I.

Table I
Solvent Dependence of $O_2(^1\Delta)$ Lifetime[a]

Solvent	$\tau_{1\Delta}$ μsec	OD_{7880}(1 cm)	OD_{6280}(1 cm)
H_2O	2	0.47	3.4
D_2O	20	0.06	0.27
CH_3OH	7	0.18	3.9
C_2H_5OH	12	0.14	2.0
C_6H_{12}	17	0.09	0.08
C_6H_6	24	0.009	0.11
CH_3CN	30	0.016	0.14
$CHCl_3$	60	0.002	0.013
CS_2	200	<0.0005	0.00
CCl_4	700	<0.0005	0.00

[a] From P. B. Merkel and D. R. Kearns, *J. Amer. Chem. Soc.*, 94, 7244 (1972).

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Stevens and Perez[31] have found that the quantum yield of DMA autoperoxidation in benzene decreases with increase in oxygen concentration in the range of 0.1 to 0.3 M (corresponding to oxygen pressures of from 200 to 600 psi) which is attributed to process 9 with an estimated rate constant of $6 \pm 4 \times 10^4$ M^{-1} sec^{-1}. Although this is considerably larger than the value for the same constant in the gas phase, it is some three orders of magnitude too low to reduce the lifetime τ_Δ to 24 μsec. A simple theoretical interpretation of process 8 in terms of electronic to (solvent) vibrational energy transfer has been presented by Merkel and Kearns[30] who relate the solvent quenching efficiency (expressed as τ_Δ) to the solvent optical densities at 7880 and 6280 cm^{-1} corresponding to the 0–0 and 0–1 components of the $O_2(^1\Delta) \rightarrow O_2(^3\Sigma)$ transition; their expression

$$1/\tau_\Delta \sim 0.5(OD_{7880}) + 0.005(OD_{6280})\ (\mu\text{sec})^{-1}$$

agrees remarkably well with the experimental values of τ_Δ listed in Table I and accommodates the tenfold increase in τ_Δ observed when D_2O (with lower optical densities at 7880 and 6280 cm^{-1}) replaces H_2O as solvent.

Photodynamic Action

Operationally defined as the photosensitized damage or modification of biological systems in the presence of molecular oxygen, photodynamic action has been extensively investigated and recently reviewed.[32] It is implicated in the spectacular effects suffered by victims of *porphyria*, an inherited disruption of porphyrin metabolism which can result in the accumulation of these potential sensitizers in the skin; in its milder form typical histamine release symptoms follow exposure of the patient to sunlight, but the proliferation of facial hair in severe cases may have proved fatal to those burnt at the stake as werewolves in the Middle Ages.[33]

Of the simpler biological systems perhaps the best documented from the kinetic standpoint is photodynamic enzyme inactivation resulting from the modification of those amino acid residues, notably histidine, tryptophan, and methionine, which are susceptible to photosensitized oxidation in the free state.[32] Since the peptide linkage is unaffected, this selective technique of key residue modification has been utilized to investigate the nature of the active enzyme site.[34] The dependence of sensitized inactivation rate on light intensity[35] and enzyme[36] (Figure 3) and oxygen[37] (Figure 4) concentration are those characteristic of the photoperoxidation reaction and logically require the consideration of $O_2(^1\Delta)$ as the reaction intermediate. Indeed, singlet oxygen has been shown to inactivate at least one (lyophilized) enzyme[38] and to react with the four major components

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(35) C. A. Ghiron and J. D. Spikes, *Photochem. Photobiol.*, 4, 907 (1965).
(36) J. D. Spikes and B. W. Glad, *Photochem. Photobiol.*, 3, 907 (1965).
(37) C. F. Hodgson, E. B. McVey, and J. D. Spikes, *Experientia*, 25, 1021 (1969).

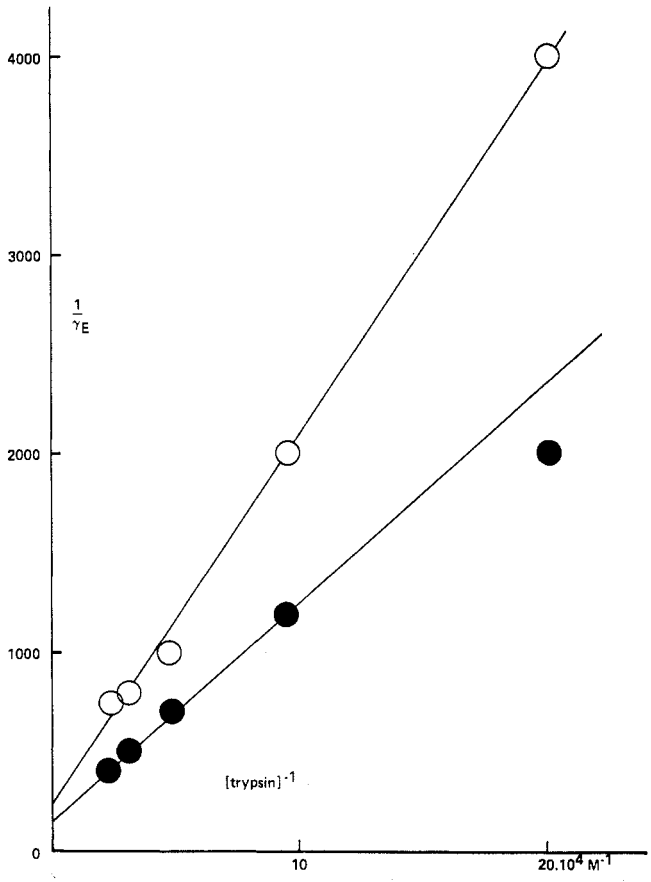

Figure 3. Photodynamic inactivation of trypsin in air-saturated aqueous solution at 15° and pH 8, illustrating typical linear dependence of γ_E^{-1} on reciprocal enzyme concentration. (○) sensitizer methylene blue (12.5 μM); (●) sensitizer FMN (150 μM). Lines drawn with same slope/intercept ratio to give $\beta = 7 \times 10^{-5}$ M. Data of Spikes and Glad.[36]

of ribonucleic acids,[39] while the rate of photosensitized oxidation of certain amino acids has been shown to increase by an order of magnitude in D_2O, reflecting a similar increase in the lifetime of $O_2(^1\Delta)$ in this solvent.[40]

From a quantitative standpoint the quantum yield γ_E of enzyme inactivation by a photoperoxidation mechanism may be represented by eq V in the form

$$\gamma_E = \gamma_{IS} \left\{ \frac{k_2{}^3\tau_S[O_2]}{1 + k_2{}^3\tau_S[O_2]} \right\} \left\{ \frac{[E]}{\beta + [E]} \right\}$$

if it is assumed that the sensitizer triplet state is produced only by intersystem crossing from the short-lived dye singlet state with efficiency γ_{IS}. The value of ${}^3\tau_S$ calculated from the oxygen concentration at which the yield is half the maximum value (Figure 4) is on the order of 10 μsec (if k_2 is assigned a spin-limited value under the stated conditions) which is characteristic of the sensitizer triplet state. Moreover, the values of β ($=k_8/k_{11}$) for enzyme inactivation computed as the slope/intercept ratios of the reciprocal yield–enzyme concentration data lines in Figure 3 are essentially independent of sensitizer

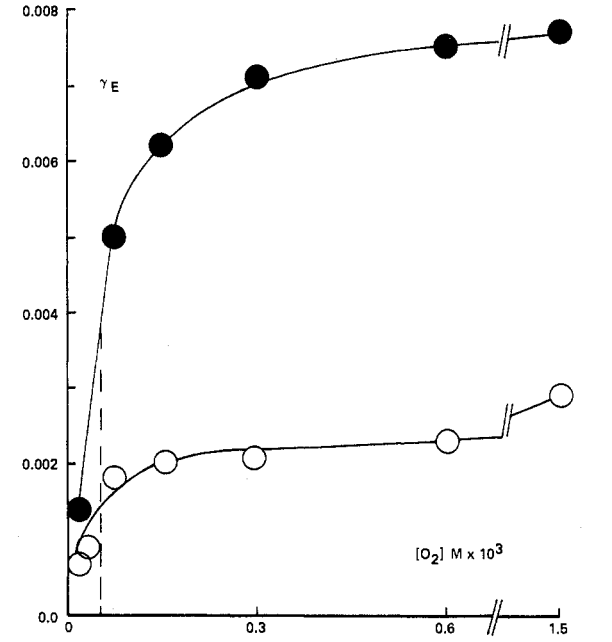

Figure 4. Variation of quantum yields for photodynamic inactivation of α-chymotypsin (40 μM) with concentration of dissolved oxygen in aqueous solution at 15° and pH 8. (○) MeB sensitizer (12.5 μM), (●) sensitized by FMN (150 μM) after Spikes, *et al.*[37]

and lie within the (albeit wide) range of those reported for photoperoxidation. It is, however, remarkable that the rate constant for reaction 11 calculat-

$$E + O_2(^1\Delta) \longrightarrow \text{inactive enzyme} \qquad (11)$$

ed, from the appropriate β-values (for trypsin) and the singlet oxygen lifetime $\tau_\Delta = 1/k_8 = 2$ μsec in water, as

$$k_{11} = 1/\tau_\Delta\beta = 7 \times 10^9\ M^{-1}\ \text{sec}^{-1}$$

should approach the diffusion-limited value for selective attack of $O_2(^1\Delta)$ on the large protein molecule; this may be indicative of sensitizer binding close to the active enzyme site.

Although these kinetic features of photodynamic enzyme inactivation are consistent with a simple photoperoxidation mechanism, others are not; thus extrapolation of the quantum yields reported for the methylene blue photosensitization of trypsin to infinite values of both enzyme and oxygen concentration provides a limiting value of $\gamma_E{}^\infty \simeq 0.005$ which is lower than the reported value of γ_{IS} for this sensitizer by a factor of ~40. Undoubtedly the reaction sequence is more complex than that indicated, due largely to the necessary use of water-soluble dyes as sensitizers which are themselves susceptible to photochemical change and which exhibit acid–base and association equilibria to introduce pH and sensitizer concentration as experimental variables. That these sensitizers play a chemical, in addition to a purely physical, role is evidenced by their consumption as the reaction proceeds;[35] indeed it appears that proteins[35] and certain amino acids[41] exercise a protective function toward sensitizer photooxidation.

(38) B. Stevens, D. G. Delgado, and J. G. Cory, Jr., *Nature (London)*, **227**, 500 (1970).
(39) F. R. Hallett, B. P. Hallett, and W. Snipes, *Biophys. J.*, **10**, 305 (1970).
(40) R. Nilsson, P. B. Merkel, and D. R. Kearns, *Photochem. Photobiol.*, in press.
(41) B. Stevens and A. Warman, unpublished.

The kinetic evidence indicates only that the overall reaction sequence involves two intermediate species with lifetimes in the microsecond region, one of which is quenched by oxygen while the second interacts with the enzyme. A strong candidate for the former is the sensitizer triplet state, which may or may not be bound to the protein, and which will react with any species present (oxygen, enzyme, or unexcited dye) to an extend depending on its concentration and the appropriate rate constant.[42] Thus the lifetime of this intermediate determined from the data in Figure 4 is on the order expected in the presence of micromolar quantities of enzyme or dye if it is determined by a diffusion-limited interaction with these species[43] rather than its (presumably much slower) unimolecular relaxation. However, since the enzyme does not inhibit the reaction, its quenching of the sensitizer triplet state must itself initiate an inactivation sequence which is stabilized by molecular oxygen at a subsequent stage. These various alternatives have been examined by Spikes and coworkers,[35] who conclude that no single exclusive mechanism can accommodate the diverse reaction characteristics.[44]

Fortunately for the victims of porphyria, speculation has not been inhibited by mechanistic uncertainty. If the symptoms of this disease are indeed provoked by photodynamic action involving a singlet oxygen intermediate, they might be expected to respond to treatment with a physical quencher of this species; the reported effects of dosage with β-carotene are little short of spectacular.[33]

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(42) C. S. Foote, *Science*, 162, 963 (1968).

(43) Measurements of the oxygen concentration dependence of quantum yield at different enzyme (and dye) concentrations would elucidate this point.

(44) Note Added in Proof: Nilsson and Kearns (*Photochem. Photobiol.*, 17, 65 (1973)) have shown that the photodynamic inactivation rates of trypsin and of alcohol dehydrogenase are increased by a factor of $\sim$10 in D_2O consistent with the increased valve of τ_Δ in this solvent (Table I) if dye and enzyme concentrations are sufficiently low to prevent their binding.