Photophysical and Photosensitized Generation of Singlet Molecular Oxygen ($^1\Delta_g$) in Micellar Solutions at Elevated Pressures. Measurement of Singlet Molecular Oxygen Solvent to Micelle Transfer Rates via Both Molecular Diffusion and Energy Transfer[1a]

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Abstract: The reaction of 1,3-diphenylisobenzofuran (DPBF) solubilized in aqueous solutions of sodium dodecyl sulfate with singlet molecular oxygen ($^1\Delta_g$) generated either continuously or by laser flash photolysis has been studied as a function of oxygen concentration. The results show that the rate constant for reaction of singlet molecular oxygen with micellar DPBF is 1.1×10^9 M^{-1} s^{-1}. The lifetimes for singlet oxygen in D_2O and H_2O are found to be 42 ± 5 and 3 ± 1 μs, respectively. The partition coefficient for molecular oxygen between the external aqueous phase and sodium dodecyl sulfate micelles is measured to be 2.8 ± 0.1. At low oxygen concentrations the transfer rate of singlet molecular oxygen from the aqueous phase to the micelle is diffusional in nature with a rate constant of 1.0×10^8 s^{-1}, with a corresponding out migration rate constant of 3.7×10^7 s^{-1}. At high concentrations of dissolved oxygen, the rate of transfer for singlet molecular oxygen across the water–micelle interface increases owing to a ground-state oxygen-mediated collisional electronic energy transfer process. The rate constants for this energy-transfer process are 9×10^9 M^{-1} s^{-1} for the inward process and 3×10^9 M^{-1} s^{-1} for the outward process.

Introduction

The term micelle[2] is used to describe a highly associated particle composed of an aggregate of surfactant monomers in aqueous solution. Micelles make a simple model for biological membranes in that both consist of hydrocarbon or lipid assemblies in an aqueous medium. Thus solution to micelle transfer rates for chemical species measured for micelles may also be applicable to biological membranes.

The transfer of oxygen molecules from the aqueous phase to membrane lipids is of particular interest because of its relevance to respiration and photodynamic inactivation[3,4] by singlet molecular oxygen.

Studies of oxygen quenching of fluorescent molecules[5,6] made soluble in solutions of aqueous micelles suggest that the micellar interior is accessible to ground-state oxygen.

A technique for making the singlet molecular oxygen acceptor DPBF water soluble by means of SDS micelles has been described by Gorman[7] et al. Reaction of micellar DPBF with singlet oxygen generated by steady-state photosensitization in the aqueous phase was observed. This technique has since been used by other investigators,[8-10] and has been extended to time-resolved systems where the singlet oxygen was generated by photosensitization[11,12] or as a result of pulse radiolysis.[13]

We wish to report a further extension where the singlet molecular oxygen is generated photophysically by Nd–YAG laser irradiation of the $^3\Sigma_g^- \rightarrow {}^1\Delta_g + 1\nu$ electronic transition of oxygen dissolved under high pressure in aqueous solution. The data is analyzed to yield singlet molecular oxygen water to micelle transfer rates occurring via both molecular diffusion and energy-transfer mechanisms.

Experimental Section

A schematic diagram of the apparatus used in these experiments is shown in Figure 1. Two high-pressure optical cells constructed from stainless steel having path lengths of 18 and 5 mm and a common aperture of 9.5 mm were used as reaction vessels in these studies. The continuous laser was a Holobeam Type 256 Nd–YAG run multimode at an average output of 1.0 W. A beam expander was used to increase the output beam diameter from 4 to 32 mm in order to completely cover the aperture of the optical cells. Laser power in the cell was determined using a Scientech Inc. Model 3600 laser power meter using a technique described previously.[14] The dye laser was a Phase-R Model 2100 B, 15-mm beam diameter, operating with the dye Kiton Red 620 at 3×10^{-5} M in methanol. The output wavelength of this dye lies with the absorption band envelope of the sensitizer methylene blue. The energy output was between 0.1 and 0.4 J as measured with a Laser Precision Corp. Model 318 pyroelectric energy meter.

Acceptor decay was measured photometrically with a collinear analyzing beam of 425 nm from a lamp and monochromator. A Corning filter CS 4-76 (Nd–YAG laser) or a specially constructed filter for maximum reflectance at 630 nm manufactured by Valpey Corp. (dye laser) were used to protect the photomultiplier in these experiments (F 2). The apparatus was protected from room light using a Kodak Wratten filter no. 88A for the Nd–YAG experiments and a Corning CS2-63 glass filter in the dye laser experiments (F 1). The output from the photomultiplier was amplified and displayed on a chart recorder (Nd–YAG) or amplified and displayed by a Tektronix 466 storage oscilloscope (dye laser). The oscilloscope input loads were 1 or 10 kΩ depending upon the bandwidth desired.

A description of the quartz iodine lamp continuous photolysis apparatus used in the photosensitized experiments can be found in ref 15.

Bio-Rad electrophoresis purity reagent grade sodium dodecyl sulfate was used in these experiments. The D_2O (99.8%) was purchased from Columbia Organic Chemical Co., Inc. The 1,3-diphenylisobenzofuran and bilirubin were supplied by Aldrich Chemical Co., Inc., and Sigma Chemical Co., respectively. Eastman white label rose bengal and Baker Analyzed reagent grade methylene blue were used in the dye-sensitized experiments. Medical grade oxygen was used for all experiments. All chemicals were used as supplied. Oxygen concentrations in solution were derived using solubility data which has been described previously.[16]

Results

The rate of reaction of micellar DPBF with singlet molecular oxygen generated photophysically is a function of both the concentrations of ground-state oxygen and surfactant. In Figure 2 the variation of rate of reaction as $k = -\mathrm{d} \ln A/\mathrm{d}t$ for DPBF in SDS micelles with singlet molecular oxygen produced photophysically by direct laser irradiation of oxygen dissolved in D_2O is shown as a function of concentration of dissolved ground-state oxygen. The results are plotted as $Nk/E[O_2]$ against O_2 where N is Avogadro's number and E is the laser

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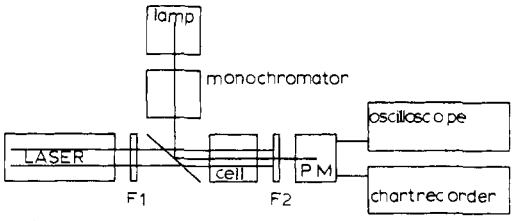

Figure 1. Schematic of apparatus.

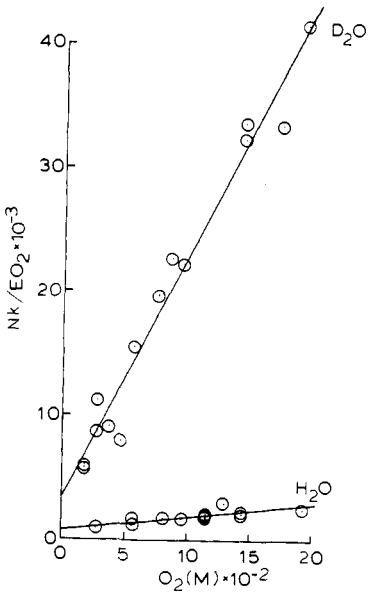

Figure 2. Reaction rate of micellar DPBF in 0.05 M SDS with photophysically generated singlet molecular oxygen as a function of dissolved oxygen concentration, 20 °C.

power ($h\nu$ s^{-1}). SDS (0.05 M) was used for all experiments (upper line neutral D_2O solution; lower line neutral H_2O solution).

Figure 3 shows the rate of reaction of DPBF in SDS micelles in D_2O with photophysically generated singlet oxygen as a function of SDS concentration at a constant concentration of ground-state oxygen, plotted as Nk/E against C_{SDS} − cmc.

If singlet molecular oxygen is generated at a constant rate by whatever method, then relative acceptor reactivities may be measured as relative rate ratios. In Table I the relative rates of reaction of micellar DPBF to bilirubin both in D_2O are compared where the singlet molecular oxygen is generated by photosensitization at low oxygen concentration and photophysically at high oxygen concentration.

The rates of reaction of micellar DPBF and bilirubin with singlet molecular oxygen generated by photosensitization are both oxygen sensitive. In Figure 4 the relative reaction rates R of both micellar DPBF and bilirubin with singlet molecular oxygen generated by continuous photosensitization are shown as a function of oxygen concentration. A constant rose bengal sensitizer concentration was used and lamp fluctuations were minimized by measuring the acceptor logarithmic decay at the O_2 concentration corresponding to atmospheric equilibration (O_2 = 0.0003 M), then pressurizing the cell to achieve a dissolved oxygen concentration of $O_2 = [O_2]$ and again measuring the reaction rate. Then R is defined as:

$$R = \frac{\text{rate at } O_2 = [O_2]}{\text{rate at } O_2 = 0.0003 \text{ M}'}$$

R_1 values corresponded to the oxygen enhancement of DPBF in SDS micelles singlet oxygen reaction rate in neutral D_2O, and R_2 to enhancement of the reaction of bilirubin in pD 8.4 D_2O solution.

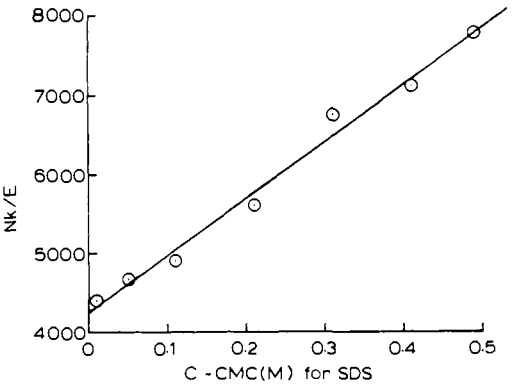

Figure 3. Reaction of micellar DPBF with photophysically generated singlet molecular oxygen at constant O_2 = 0.146 M, 20 °C, as a function of SDS concentration.

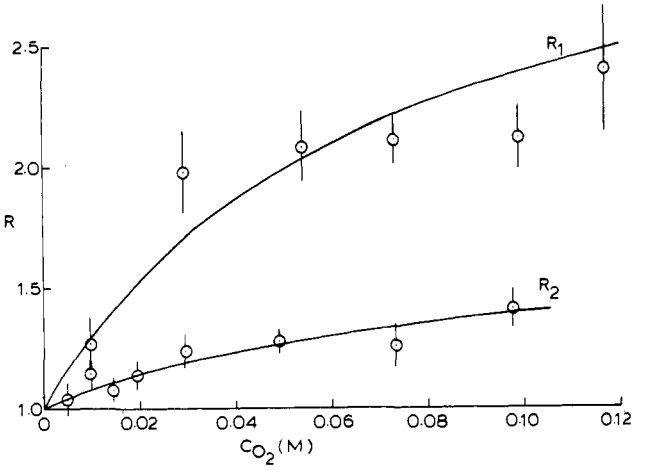

Figure 4. Relative rates of reaction of DPBF in 0.1 M SDS in D_2O and bilirubin in pD 8.4 D_2O with singlet molecular oxygen generated by rose bengal photosensitization as a function of dissolved oxygen concentration, 20 °C.

Table I. Rate Ratio Comparisons for Micellar DPBF, 0.1 M SDS, pD Neutral, and Bilirubin in pD 8.4 D_2O at 20 °C[a]

	photosensitized O_2 = 0.0003 M	photophysical O_2 = 0.195 M
k_{DPBF}/k_{bil}	2.3 ± 0.5	7.5 ± 0.7

[a] Photosensitization constant light intensity 550 nm selected with Wratten 54 filter and constant concentration of rose bengal, photophysical constant Nd laser power, and 0.195 M O_2.

Since the rate of reaction of micellar DPBF with singlet molecular oxygen generated by continuous photosensitization is a function of oxygen concentration, it was decided to study the reaction of micellar DPBF with a concentration pulse of singlet molecular oxygen generated by laser flash photosensitization. The method in effect measures the decay of the singlet molecular oxygen concentration pulse, and has the advantage that absolute chemical reaction rate constants and singlet molecular oxygen lifetimes may be obtained without knowledge of absolute singlet molecular oxygen concentrations.

In Figure 5 redrawings of experimental oscilloscope traces are shown for experiments where DPBF in SDS micelles was reacted with a concentration pulse of singlet molecular oxygen produced by dye laser flash photolysis of the sensitizer methylene blue. The upper curve shows the reaction in the presence of 0.122 M oxygen, and the lower in the presence of 0.003 M oxygen.

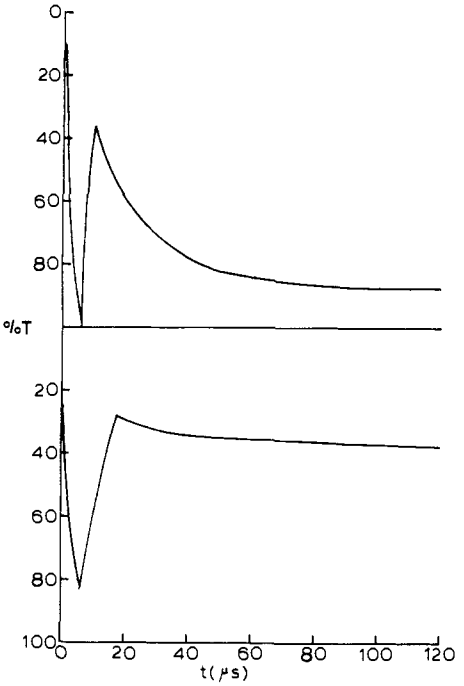

Figure 5. Experimental oscilloscope traces for the reaction of micelles DPBF in 0.1 M SDS with singlet molecular oxygen produced by laser flash photosensitization for two different dissolved oxygen concentrations, 20 °C. Note transmission increases in downward direction.

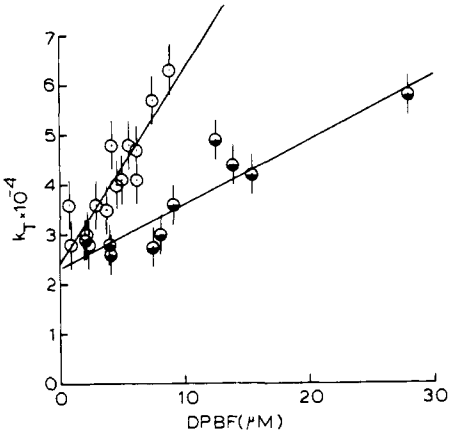

Figure 6. Variation of total decay rate of singlet molecular oxygen produced by laser flash photosensitization with concentration of micellar DPBF for two different oxygen concentrations, 20 °C.

The singlet molecular oxygen concentration pulse decays shown in Figure 5 may be analyzed in terms of a total decay rate, k_T, which is a function of DPBF concentration. (A detailed kinetic analysis will be given in the Discussion section.) Figure 6 shows the variation of the total decay rate of singlet molecular oxygen, k_T, with the concentration of micellar DPBF in D_2O solution (upper line for 0.122 M oxygen, lower for 0.0003 M oxygen); both lines contain the data of Figure 5 and additional experiments.

k_R values, representing the total chemical and physical reaction rate constant of DPBF with singlet molecular oxygen, may be obtained from the slopes of the lines shown in Figure 6. These k_R values and others for different oxygen concentrations are shown in Figure 7 as a function of oxygen concentration. The curve used to fit the data is derived in the Discussion section.

Figure 8, lower curve, shows the measured decay rate of micellar DPBF in D_2O upon reaction with a concentration pulse of singlet molecular oxygen of unusually high concentration. The upper curve shows the estimated concentration of singlet molecular oxygen and its decay with time. The derivation of this curve is described in detail in the Discussion section.

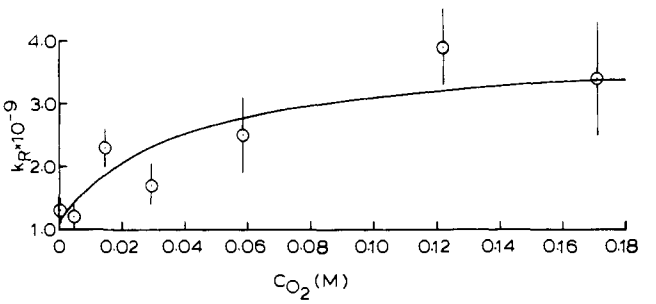

Figure 7. Variation of micellar DPBF singlet molecular oxygen chemical reaction rate constants as determined by laser flash photolysis with concentration of dissolved oxygen, 20 °C.

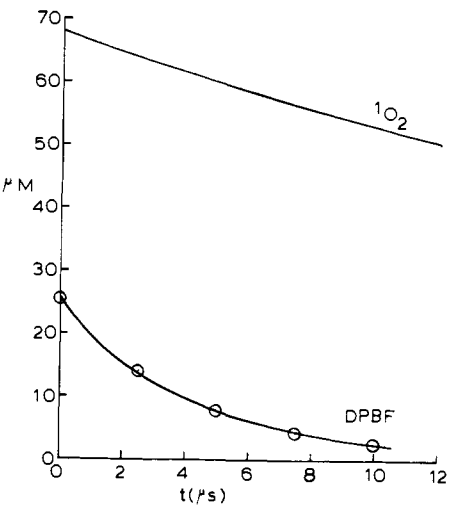

Figure 8. Reaction of micellar DPBF in 0.1 M SDS with singlet molecular oxygen at high singlet molecular oxygen concentration. Lower curve decay of DPBF (measured), upper curve decay of singlet molecular oxygen (estimated) ($[O_2]$ = 0.195 M, methylene blue 30.8 M, and 0.4 J laser energy).

Discussion

There are two aspects of the results of this work that require explanation. The first is the effect of surfactant concentration at constant oxygen concentration and Nd laser power on the experimental reaction rates. The second effect is the enhancement of rates with oxygen concentration seen in various experiments.

Effect of Surfactant Concentration. The effect of surfactant concentration may be analyzed as follows. The absorption at 1.06 μm O_2 ($^3\Sigma_g^-$) + O_2 ($^3\Sigma_g^-$) + $h\nu \rightarrow O_2$ ($^1\Delta_g$) + O_2 ($^3\Sigma_g^-$) leading to singlet molecular oxygen is collisional in nature and thus proportional to the square of oxygen concentration. This has been shown by analysis of bilirubin photochemical experiments in D_2O[15] where the rate is almost entirely due to the square of oxygen concentration with a small linear component. This is confirmed by the results of Figure 2 of this work and the absorption will be approximately proportional to $[O_2]^2$ at high oxygen concentrations.

Let us consider a solution consisting of an aqueous phase and a micellar phase only, so that, if the oxygen concentration is $[O_2]$ in water, it is $K[O_2]$ in the micellar phase where K is the distribution coefficient giving the concentration of oxygen in the micellar phase relative to the aqueous phase. Thus since the photophysical production of singlet molecular oxygen is

proportional to $[O_2]^2$ and given a micelle volume fraction f the rate of production of Δ will be given by

$$d(\Delta)/dt = \sigma E/N[K^2[O_2]^2 f + (1-f)[O_2]^2]$$
$$= (\sigma E/N)[O_2]^2[(K^2-1)f + 1]$$

where σ is the oxygen absorption cross section at 1.06 μm and E/N is the laser power in mol $h\nu$ s^{-1}.

At low DPBF concentrations the dominant loss process for Δ is solvent deactivation at a pseudo-first-order decay rate given by $1/\tau$, and the steady-state oxygen concentration will be given by

$$[\Delta] = (\sigma E/N)[O_2]^2\tau[(K^2-1)f + 1]$$

The rate of disappearance of A is given by

$$-d(\ln A)/dr = k_1 = k_A[\Delta]$$
$$= \sigma\tau(E/N)[O_2]^2 k_A[(K^2-1)f + 1]$$

At constant oxygen concentration C and laser power E we may write

$$k_1 = \text{const}[(K^2-1)f + 1]$$

The volume fraction of micelles f is given by $f = \bar{v}(C_{SDS} - \text{cmc})$ where $\bar{v}$ is the partial molar volume of SDS = 0.2462 L[17] and then

$$k_1 = \text{const}[(K^2-1)\bar{v}(C_{SDS} - \text{cmc}) + 1]$$

The data of Figure 3 have been fitted to such an equation and yield slope/intercept = 1.69 ± 0.13 which, substituting for $\bar{v}$, yields $K = 2.8 \pm 0.1$. This is in good agreement with the value of 2.9 for the partition coefficient of oxygen between micelles and water previously obtained in this laboratory by a more direct method.[16] The agreement also suggests that the assumption that the oxygen optical absorption in D_2O approximates to $(O_2)^2$ is adequate at reasonably high oxygen concentrations.

The fact that at constant E and O_2 the data can be analyzed without knowledge of the absolute value of either makes this photochemical technique attractive for estimation for other surfactant solvent systems.

Effect of High Oxygen Concentrations on Experimental Rates. The data of Table I where the relative reaction rates of DPBF in SDS micelles to those for bilirubin under conditions of photosensitization and low oxygen concentration are compared to those obtained using photophysical oxygen generation show an enhancement in the ratio on going to high oxygen concentrations. If it may be assumed that the rate constant for chemical reaction of bilirubin with Δ oxygen is not affected by oxygen concentration, then the rate of reaction of micellar DPBF with Δ must increase with increasing oxygen concentrations. The ratio of rate ratios (k_{DPBF}/k_{bil})high O_2: (k_{DPBF}/k_{bil})low O_2 suggests that k_{DPBF}(high O_2)/k_{DPBF}(low O_2) = 3.3 ± 1.0. This value is rather similar to the 2.8K value found above in the variation of rate with SDS concentration experiments described above. The question becomes: how does this rate enhancement arise?

Electronic energy transfer of $^1\Delta_g$ excitation in the gas phase has been demonstrated by the experiments of Jones and Bayes,[18] who combined a photoionization detection technique and isotopic substitution to detect excitation transfer from one isotopic oxygen molecule to another. The energy transfer occurred at least once in ten collisions and proceeded via an electron-exchange mechanism. The phenomenon has since been invoked to explain some features of $^1\Delta_g$ oxygen and triplet organic chromophore annihilation fluorescence[19] and the deactivation of laser-generated $^1\Delta_g$ oxygen by ground-state oxygen in the gas phase.[20] A similar energy transfer for $^1\Sigma_g^+$ oxygen in isolated dimers has been observed by Goodman and Brus.[21]

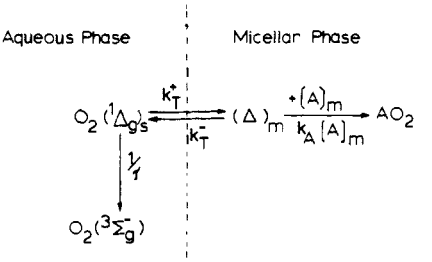

Figure 9. Schematic of a water–micelle interface showing proposed rate processes.

It is possible to envisage such an energy-transfer process taking place in solution where the oxygen concentration is high enough so that collisions of Δ with ground-state oxygen have a reasonable probability.

The rate constants observed experimentally depend upon to what extent the energy transfer across the water–micelle interface competes with normal molecular diffusion across the interface. A schematic diagram of a micelle–water interface is shown in Figure 9. It is assumed that the Δ oxygen is generated entirely in the aqueous medium and the sole decay route other than reaction with A is quenching by the solvent. It is further assumed that there is negligible quenching due to the micellar material. Then, if Δ is generated at a rate R and has a solvent-mediated lifetime of τ, the Δ steady state in the solvent is given by $[\Delta]_s = R\tau$. The steady state in the micelle will be given by the rate at which the Δ enters the micelle, $k_T^+[\Delta]_s$, divided by the rate at which it leaves the micelle, the sum of out-migration and reaction with A.

$$[\Delta]_M = \frac{k_T^+[\Delta]_s}{k_T^- + k_A[A]_M}$$

$[A]_M$ is the concentration of [A] in the micelle seen by $[\Delta]_M$. Since [A] is typically of the order of 10^{-5} M and C micelle 10^{-3} M, only a small fraction of the micelles will be occupied and those occupied will be overwhelmingly singly occupied. Then the concentration of A in the micelle, $[A]_M$, will be given by the reciprocal of the micelle molar volume, $\bar{v}_M = \bar{v}_{SDS}\,\bar{n}$, where $\bar{n}$ is the mean aggregation number for SDS micelles. Literatures values for $\bar{v}_{SDS}$[17] and $\bar{n}$[22] yield $[A]_M$ = 0.065 M.

The rate of disappearance of A as measured experimentally will be given by

$$\frac{-1}{[\Delta]_s}\frac{d\ln A}{dt} = k = \frac{k_A k_T^+}{k_T^- + k_A[A]_M}$$

where A represents the bulk concentration of A. While the terms k_A and $[A]_M$ are constant, the proposed mechanism requires that k_T^+ and k_T^- increase with increasing oxygen concentration. Where $k_T^+, k_T^- \gg k_A[A]_M$, it may be shown that

$$k = k_A k_T^+/k_T^- = k_A K$$

This equation assumes that the same partition coefficient is established by both molecular diffusion and energy transfer. At low oxygen concentrations, provided that k_T^+ and k_T^- are low enough, k goes to the limit $k = k_T^+/[A]_M$. It is interesting that, if this model is correct and since the experimental rate enhancement due to oxygen $\simeq K$, $k_A \simeq k_T^+/[A]_M$. This coincidence of numerical values was initially misleading since it appeared that at low oxygen concentrations k_A was being measured.

The equation may be rearranged to give

$$k = \frac{Kk_A}{1 + (k_A/k_T^-)[A]_M}$$

The proposed mechanism requires that the transfer rate in-

creases with oxygen, and the simplest expression describing this is

$$k_{\text{transfer}}^{-} = k_T^{-} + k_E^{-}[O_2]$$

where k_E^{-} is the out-migration rate due to energy transfer and $[O_2]$ is the oxygen concentration in the solvent. Thus the equation describing the experimental rate k becomes

$$k = \frac{Kk_A}{1 + \dfrac{k_A[A]_M}{k_T^{-} + k_E^{-}[O_2]}}$$

The question may now be raised as to whether the Δ oxygen has a sufficiently long lifetime for the concentration partitioning in the micelles to occur. The required time is in fact rather short since given an oxygen (excited assumed equal to ground) molecular diffusion coefficient $D = 2 \times 10^{-5}$ cm^2 s^{-1} [23] only 25 ns is required to traverse the intermicellar separation of 100 Å (for 0.1 M SDS). Since the Δ lifetime is of the order of 40 μs each Δ will have the opportunity to collide with many micelles within its lifetime and micelle concentration partitioning can occur. This will be true for all the experimental studies of this work where the observation time is always greater than or equal to the time required for oxygen partitioning.

The experimental evidence discussed so far has been a comparison of reaction rates for photophysically generated singlet oxygen (high ground-state oxygen) with singlet oxygen generated by photosensitization at low oxygen concentrations. The data to be discussed now concerns photosensitization under conditions of high oxygen concentration.

Effect of Oxygen Concentration for Continuous Photosensitization Rates. The variation of experimental reaction rate ratio R (rate at oxygen concentration of $[O_2]$ to that for $[O_2] = 0.0003$ M, the concentration achieved at equilibrium with air) as a function of oxygen concentration is shown in Figure 4. In the case of DPBF in 0.1 M SDS, R_1, there is an appreciable increase in R_1 with oxygen. Only part of this enhancement is due to a micellar effect since a similar but smaller enhancement is seen for the rose bengal photosensitized reaction of the singlet oxygen acceptor bilirubin, R_2. The increase in R_2 is due to oxygen-induced intersystem crossing in the photosensitizer, and a kinetic analysis is given below.

The following processes govern the triplet yield of the photosensitizer.

$S_0 \xrightarrow{h\nu} {}^1S$ absorption rate $\sigma E[S]$

${}^1S \rightarrow S_0 + h\nu$ fluorescence, rate constant k_2

${}^1S \rightarrow S_0$ internal conversion, rate constant k_4

${}^1S \rightarrow {}^3S$ intersystem crossing rate constant k_3

${}^1S + {}^3O_2 \rightarrow {}^3S + {}^3O_2$ oxygen-induced intersystem crossing rate constant k_x

In the absence of O_2

$$\phi_T = \frac{k_3}{k_2 + k_3 + k_4}$$

In the presence of O_2 at concentration $[O_2]$

$$\phi_T' = \frac{k_3 + k_x[O_2]}{k_2 + k_3 + k_4 + k_x[O_2]}$$

If $\phi_T'/\phi_T = R$, it may be shown that

$$R = \frac{1 + (k_x/k_3)[O_2]}{1 + \dfrac{k_x[O_2]}{k_2 + k_3 + k_4}} = \frac{1 + a[O_2]}{1 + b[O_2]} \text{ and } \phi_T = b/a$$

The above equations show how the sensitizer triplet yield is enhanced by oxygen. Given the triplet yield and a constant rate of illumination E the singlet oxygen generation rate will be $\sigma E[S]\phi_T\phi_{O_2}$, where ϕ_{O_2} is the quantum yield of singlet oxygen production from the triplet, and will closely approach unity for all appreciable oxygen concentrations. Thus for an acceptor reactivity index $\beta = 1/\tau k_A$, it may be shown that the rate of reaction is given by

$$-\frac{d \ln A}{dt} = \frac{\sigma E[S]\phi_T\phi_{O_2}}{N\beta}$$

Under conditions of constant illumination intensity and sensitizer concentration this simplifies to

$$-d \ln A/dt = \text{const}\phi_T$$

Thus for bilirubin R_2 will be the function of $[O_2]$ given above. If the R_2 curve is analyzed by a least-squares method, $a_2 = 20 \pm 6$ and $b_2 = 11.5 \pm 5$, leading to $\phi_T = 0.6 \pm 0.4$. This ϕ_T value is in agreement with the 0.76 value of Gollnick and Schenck.[24] It is likely that this oxygen induction technique for determining triplet yields will be of greater precision and utility for the determination of triplet yields nearer to zero than unity, where the oxygen enhancement will be correspondingly more marked. It is also possible to estimate k_x from the present data. Gollnick and Schenck give $k_3 = 1.73 \times 10^9$ M^{-1} s^{-1}, which when combined with $a_2 = 20 \pm 6$ yields $k_x = 3.5 \pm 1 \times 10^{10}$ M^{-1} s^{-1}. This value for the rate constant of oxygen-induced intersystem crossing is approximately the diffusion-controlled limit and suggests that induced intersystem crossing is very efficient, occurring on every oxygen excited singlet dye collision within margin of error.

The data for DPBF in SDS micelles R_1 was also fitted to an empirical curve of the form

$$R_1 = \frac{1 + a_1[O_2]}{1 + b_1[O_2]}$$

This yields the coefficients $a_1 = 49 \pm 19$, $b_1 = 15 \pm 9$. The R_1 values represent two effects: oxygen-induced intersystem crossing and oxygen enhancement of the rate of reaction of micellar DPBF with Δ oxygen. The curve for micellar DPBF rises much more rapidly with increasing oxygen concentration than that for bilirubin, the enhancement (relative to bilirubin) reaching a value of 1.8 at the maximal oxygen concentration of the experiments. While this is a large increase, it is not quite as high as the 3.3 value obtained from Table I, and the support is only partial.

Effect of Oxygen Concentration on Flash Photosensitization Rates. The flash photolysis experiments of Figures 5 and 6 are also supportive of an excitation energy transfer mechanism leading to rate enhancement.

This method has an advantage over steady-state photosensitization in that the decay of a Δ concentration pulse is observed, and that knowledge of absolute concentrations is not required so that changes in sensitizer triplet yield are of no consequence. The concentration pulse of Δ oxygen is produced by energy transfer from the sensitizer in less than 1 μs after the 200-ns dye laser pulse. The rates of decay of Δ and the acceptor A are given by the equations

$$-dA/dt = k_R[A][\Delta] \quad \text{(i)}$$

$$-d\Delta/dt = (1/\tau + (k_R + k_Q)[A]) \quad \text{(ii)}$$

The usual assumption is $[A] > [\Delta]$[25,27] so that $[A]$ does not change appreciably. The decay of Δ is given by (ii), solved as

$$\ln [A - A_\infty] = [1/\tau + (k_R + k_Q)[A]]t + \text{const} \quad \text{(iii)}$$

This expression has been shown by Young et al.[26] to be approximately true under conditions where $[A] \simeq [\Delta]$ and the

change in [A] is appreciable, provided that $[\overline{A}]$ is used for [A] in (iii).

The [A] dependent term in (iii) has terms for chemical reaction k_R and physical quenching k_Q. It is assumed in the following analysis that $k_R > k_Q$ and k_Q is neglected.

The redrawings of experimental oscilloscope traces shown in Figure 5 show a strikingly greater photobleaching of DPBF in the case where 0.122 M oxygen is present. It has been shown by Merkel and Kearns[25] that the maximum photobleaching occurs for minimum values of the reactivity index $\beta = 1/\tau k_R$ and thus one would expect that k_R is much greater in the experiment where 0.122 M oxygen is present. This is indeed the case, and is shown in Figure 6, where solutions of (iii) or total rate $k_T = 1/\tau + k_R[A]$ are plotted for 0.122 oxygen and 0.0003 M oxygen (concentration achieved in D_2O solution at equilibrium with air). Least-squares analysis gives from the intercepts:

$$[O_2] = 0.0003\ M \qquad \tau = 43 \pm 5\ \mu s$$

$$[O_2] = 0.122\ M \qquad \tau = 41 \pm 5\ \mu s$$

These lifetime values in D_2O are not significantly different from the 35-μs value derived from bilirubin studies[15] and the 32-[11] and 30-μs[12] values derived from earlier DPBF in aqueous SDS studies. The range of values probably represents the large imprecision inherent in such laser flash photolysis lifetime experiments.

The slopes of Figure 5 yield

$$[O_2] = 0.0003\ M \qquad k_R = (1.3 \pm 0.2) \times 10^9\ M^{-1}\ s^{-1}$$

$$[O_2] = 0.122\ M \qquad k_R = (3.9 \pm 0.6) \times 10^9\ M^{-1}\ s^{-1}$$

The $1.3 \pm 0.2 \times 10^9\ M^{-1}\ s^{-1}$ value for $k_T^-/[A]_M$ at low oxygen concentration is similar numerically to earlier DPBF reaction rate values in aqueous SDS studies[11] and DPBF chemical reaction rates in other solvents[25-28] all of which are in the vicinity of $10^9\ M^{-1}\ s^{-1}$. The value obtained in this study at 0.122 M oxygen of $3.9 \pm 0.6 \times 10^9\ M^{-1}\ s^{-1}$ is about three times higher than the low oxygen value, and is interpreted as an apparently higher reaction rate constant being observed at high oxygen concentrations, i.e.

$$k_R = Kk_A$$

This further suggests that Δ equilibrium to give a higher concentration in the micelles must occur much more rapidly than the microsecond time scale of the laser flash photolysis experiments. Only two decay rates for two oxygen concentrations are shown in Figure 6 in the interest of clarity. Similar rates were measured for other oxygen concentrations and are shown as a k vs. O_2 plot in Figure 7. The standard deviations for each k determination are indicated by the depth of the vertical bars and the curve fitted is

$$k = \frac{Kk_A}{1 + \dfrac{k_A[A]_m}{k_T^- + k_E^-[O_2]}} \qquad \text{i.e., } k = \frac{Kk_A}{1 + \dfrac{1}{a + b[O_2]}}$$

where

$$a = k_T^-/k_A[A]_M \text{ and } b = k_E^-/k_A[A]_M$$

The values used are $Kk_A = 3.9 \times 10^9$, $a = 0.41$, and $b = 34.5$, yielding for $[A]_M = 0.065$ M, $k = 2.8$, $k_A = 1.4 \times 10^9\ M^{-1}\ s^{-1}$, $k_T^- = 3.7 \times 10^7\ s^{-1}$, $k_T^+ = 1.0 \times 10^8\ s^{-1}$, $k_E^- = 3.2 \times 10^9\ M^{-1}\ s^{-1}$, and $k_E^+ = 9 \times 10^9\ M^{-1}\ s^{-1}$. The k_A value of $1.4 \times 10^9\ M^{-1}\ s^{-1}$ for the chemical reaction rate constant of DPBF with Δ, although on the high side, is compatible with values measured in other solvents.[25-28]

The k_T^- of $3.7 \times 10^7\ s^{-1}$ for the diffusional out-migration of oxygen has not been previously measured. Out-migration rates have been measured for relatively large molecules by phosphorescence[5] and EPR of spin-labeled compounds,[29,30] and yield values of $\simeq 10^5\ s^{-1}$.

The most comparable measurements are those of Yiv and Zana[31] for 1-pentanol leaving aqueous cetyltrimethylammonium bromide micelles, who measured k_T^- to be $1.8 \times 10^7\ s^{-1}$. The twofold increase in out-migration rate in going from pentanol to oxygen is in reasonable agreement with the variation of known diffusion rates in water as a function of molecular weight.[23]

The measured energy transfer excitation transfer rates k_E^+ and k_E^- are about an order of magnitude lower than the diffusion-controlled limit of 10^{10}–10^{11} for a small molecule in solution which suggests that a Δ molecule at the micelle solvent interface has a probability of 0.1 of encountering a ground-state oxygen molecule and transferring its energy. The rough nature of the experimental results and the simplicity of the model do not permit a more detailed mechanistic discussion. All of the experiments shown in Figure 7 were carried out using 0.1–0.2 J of laser energy at 620 nm and methylene blue concentrations of 2.6 or 7.7 M. Under these conditions $[\Delta] \leq$ [DPBF] and solutions of (ii) will hold.

It also has proved possible to carry out experiments where $[\Delta] \gg$ [DPBF] and where (i) applies. These results are shown in Figure 8, where 0.4 J laser power, 30.8 μM methylene blue, and 0.195 M oxygen were used. A precisely first-order decay rate of $2.3 \times 10^5\ s^{-1}$ was observed for DPBF (lower curve); then, if $-d(\ln A)/dt = k_1 = k_R[\Delta]$ is solved for Δ assuming $k_R = 3.9 \times 10^9\ M^{-1}\ s^{-1}$ (the largest experimental value), $[\Delta] = 60\ \mu M$. The upper curve shows that 60-μM Δ concentration centered at 5 μs with a lifetime of 40 s superimposed. It is clear that the concentration of A is insufficiently high to appreciably affect Δ. The result is remarkable in one other respect. The concentration of Δ generated by the laser pulse (>60 μM) is twice the initial methylene blue concentration. This arises for two reasons. Firstly, the high ground-state oxygen concentration drives the unperturbed methylene blue triplet yield from 0.52[32] to unity by oxygen-induced intersystem crossing; secondly, the high ground-state oxygen concentration causes rapid (5 ns) quenching of methylene blue triplets so that the methylene blue may be recycled within the 180-ns measured time period of the laser pulse. This multiple sensitizer excitation phenomenon is unusual and arises because of the elevated oxygen concentrations unique to this study.

The high rate constant at high O_2, Kk_A has made it possible to carry out flash photolysis experiments in the H_2O where τ is much lower, since the maximum photobleaching is proportional to $1/\beta = Kk_A\tau$ and, although Δ is lower, the rate constant is K times higher than that at low O_2. The cells available were not short enough to permit use of [A] values high enough to obtain a significant estimate of Kk_A ($Kk_A[A] < 1/\tau$). However, it has been shown[28] that $1/\tau$ values may be obtained as $[A] \to 0$, i.e., $k_T = 1/\tau$. The mean of many such experiments for 0.146 M O_2, 7.7 μM Meb, and 0.3 J laser energy yielded $\tau_{H_2O} = 3 \pm 1$ μs. Thus, given $\tau_{D_2O} = 42 \pm 5$ μs as the mean value from the D_2O experiments above

$$\tau_{D_2O}/\tau_{H_2O} = 14 \pm 6$$

This agrees within experimental error with the literature value[27] of 10 obtained from water-alcohol mixed solvent studies.

Analysis of Photophysically Generated Singlet Molecular Oxygen Reaction Rates. All the foregoing evidence shows that at high concentrations of ground-state oxygen the energy-transfer mechanism of Δ excitation can occur and the effective Δ concentration is K times higher inside the micelle relative to the solvent. Given this information it is now possible to analyze the DPBF reaction rate as a function of oxygen concentration with photophysically generated single oxygen depicted in Figure 1.

Table II

	$[^3O_2]$	$[^1O_2]$	type of expt	derived constants	value
regime I	low 10^{-4}	low 10^{-11}	sensitized continuous	$k_T^+/[A]_M$ or rel B	$\tau_{D_2O} = 42 \pm 5\ \mu s$
		10^{-5}	sensitized pulse	$1/, k_T^+/[A]_M$	$k_T^+ = 1 \times 10^8\ s^{-1}$
					$k_T^- = 3.7 \times 10^7\ s^{-1}$
regime II	high 10^{-1}	10^{-11}	sensitized continuous	Kk_A or rel B	$K = 2.8$
					$k_A = 1.1 \times 10^9\ M^{-1}\ s^{-1}$
		low 10^{-11}	photophysical continuous	Kk_A, K	$k_E^+ = 9 \times 10^9\ M^{-1}\ s^{-1}$
		10^{-5}	sensitized pulse	$1/, Kk_A$	$k_E^- = 3.2 \times 10^9\ M^{-1}\ s^{-1}$
regime III	high 10^{-1}	high 10^{-4}	sensitized pulse	$Kk_A[\bar{\Delta}]$	$[\bar{\Delta}] = 10^{-4}$ M

This type of plot is very similar to that observed for the reaction of photophysically generated singlet oxygen with bilirubin,[15] i.e., for both D_2O and H_2O solvents the effect is largely square with a small but significant linear component and the experimental reaction rates are approximately an order of magnitude higher in D_2O than in H_2O. Although an increase in the experimental rate due to oxygen-induced energy transfer is to be expected in the low oxygen concentration region, no such change is visible in Figure 2. The experimental scatter is too large to permit curvature analysis at low oxygen concentrations, and a straight line corresponding to the high oxygen concentration limit $k_R = Kk_A$ has been fitted to the data.

Given that $[\Delta]$ is generated at a rate given by

$$\frac{+d[\Delta]}{dt} = \frac{\sigma_1 E[O_2]}{a} + \frac{\sigma_2 E[O_2]^2}{a}$$

it may be shown that

$$Nk_1/E[O_2] = (\sigma_1/a)\tau K k_A + (\sigma_2/a)\tau K k_A[O_2]$$

From the D_2O data of this work

$$\text{intercept} = \sigma_1 \tau K k_A/a = (3.3 \pm 1.1) \times 10^3\ dm^3\ mol^{-2}$$

$$\text{slope} = \sigma_2 \tau K k_A/a = (1.96 \pm 0.11) \times 10^5\ dm^6\ mol^{-3}$$

The earlier bilirubin in D_2O results[15] gave $\sigma_1\tau = (2.3 \pm 0.8) \times 10^{-8}$ s dm^2 mol^{-1} and $\sigma_2\tau = (4.6 \pm 1.2) \times 10^{-7}$ s dm^5 mol^{-2}. The cell used has a diameter of 9.51 mm so that $a = 7.13 \times 10^{-3}$ dm^2. Considering the slope term only in D_2O since it is much better determined, then

$$Kk_A = \frac{\sigma_2 \tau K k_A}{a}(a/\sigma_2\tau) = (3 \pm 1) \times 10^9\ M^{-1}\ s^{-1}$$

This value is in good agreement with the asymptotic Kk_A value of 3.9×10^9 M^{-1} s^{-1} obtained from the dye-sensitized flash photolysis experiments and suggests that the hypothesis of preferentially partitioning in the micelles via excitation energy transfer is consistent with all of the experimental data. The rate constants observed are dependent on the oxygen concentrations (both ground and excited) and are summarized in Table II.

In conclusion, the present study shows that singlet molecular oxygen water to micelle transfer rates via both molecular diffusion and bimolecular energy transfer process may be derived from photochemical measurements of singlet molecular oxygen reactions. The value of the molecular diffusion transfer rate, 10^8 s^{-1}, suggests that singlet molecular oxygen penetrates SDS micelles easily at a rate greatly exceeding its solvent-mediated decay rate, 3×10^5 s^{-1} in water. Insofar as micelles are simple models of biological membranes it appears that the interior of membranes will also be easily accessible to singlet molecular oxygen which can then cause photodynamic damage to material in the membranes.

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References and Notes

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