Reactions of Singlet Oxygen with Olefins and Sterically Hindered Amine in Mixed Surfactant Vesicles

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Received May 24, 1999. Revised Manuscript Received November 5, 1999

Abstract: The photosensitized oxidations of the olefins, *trans*-1,2-dimethoxystilbene (DMOS), *trans*-stilbene (TS), and *trans,trans*-1,4-diphenyl-1,3-butadiene (DPB) as well as the amine 2,2,6,6-tetramethylpiperidine (TMP) in mixed surfactant vesicles were investigated. The sensitizer was either a hydrophobic dye, tetraphenylporphyrin (TPP), or a cationic dye, methylene blue (MB). The substrate molecules were solubilized in the bilayer membranes of one set of vesicles, and the sensitizers were incorporated in the bilayers or the aqueous inner compartments of another set of vesicles. The irradiation samples were prepared by mixing the above two sets of vesicle dispersions. Photoirradiation of the oxygen-saturated samples resulted in the oxidation of the substrates, as evidenced by the isolation of the end products in the olefin oxidation and by the detection of the ESR spectrum of the nitroxide radical in the amine oxidation. The quantum yields for the product formation were enhanced significantly in D_2O dispersions compared with those in H_2O medium. All of these observations suggest that singlet oxygen generated in the bilayer or the inner water pool of one vesicle is able to diffuse out and enter into the bilayer of another vesicle through the aqueous dispersion and react with the target molecules. The measurements of the quantum yields revealed a substantial fraction of the singlet oxygen diffusing from its generated locus to the reaction sites: 8% in H_2O and 15% in D_2O dispersions in the case of singlet oxygen generated in the inner aqueous compartment of the vesicle; 20% in H_2O and 80% in D_2O dispersions for the singlet oxygen generated in the bilayer of the vesicle. In the photosensitized oxidation of TS and DPB in vesicles, the 1,2-cycloaddition products of singlet oxygen to the olefins were detected in quantitative yields, which was in sharp contrast to the oxidation in homogeneous solutions where the 1,4-cycloaddition products of the singlet oxygen to the dienes were the unique products. This result indicates that the organized semi-rigid environment in vesicles prevents the olefins from conformation change.

Introduction

Photoprocesses in vesicles have been extensively investigated.[1] Much of the interest in such studies originates from possible analogies between these processes and phenomena occurring in biological systems, particularly in membranes and related structures. The coexistence of an amphiphilic bilayer and an aqueous compartment in vesicle composes a microheterogeneous system that mimics specific situation occurring at the cell level. Of particular interest to photochemists and photobiologists are the photosensitized oxidation reactions since they are related to photodynamic reactions.[2] It has been established that in many cases photodynamic process involves production and subsequent reaction of singlet oxygen ($^1O_2$), although other reasonable alternative mechanisms including free radical formation and superoxide also have been proposed.[3] This process is of extremely complex nature because it involves several steps, and the factors determining its rate (such as the locus of $^1O_2$ generation, $^1O_2$ diffusion rate,$^1O_2$ partition between different environments, and $^1O_2$ lifetime) are influenced by environment. To gain a better understanding of photodynamic effects, the photosensitized oxidations of various substrates incorporated within vesicles have been investigated.[1a] The central point addressed in most of such studies is whether singlet oxygen generated intravesicularly reacts inside the same vesicle or with target molecules located in other vesicles, and the results have been frequently contradictory. For example, Dearden[4]

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10.1021/ja9917161 CCC: $19.00
Published on Web 03/04/2000

estimated that the deactivation of $^1O_2$ generated by the membrane-bound sensitizer occurred efficiently (90%) in the lipid layer of the lecithin liposomes. Hoebeke et al.[5] also concluded that with a sensitizer bound to dimyristoyl phosphatidylcholine liposomes, $^1O_2$ spent more than 87% of its lifetime in the liposome environment. On the other hand, Reddi,[3g] Kanofsky,[6] Ehrenberg,[2c] Grossweiner,[3f,7] Rodgers,[8] Nonell,[9] and their co-workers concluded that a significant fraction of the $^1O_2$ generated by liposome-bound sensitizer escapes from the lipid bilayer to the water phase and there exists fast exchange of $^1O_2$ between vesicles and aqueous solution and even among vesicles.

In the present paper, we report the results of a study of photosensitized oxidations of three olefins and a sterically hindered amine in mixed surfactant vesicles. The olefins we studied were *trans*-1,2-dimethoxystilbene (DMOS), *trans*-stilbene (TS), and *trans*,*trans*-1,4-diphenyl-1,3-butadiene (DPB), and the amine was 2,2,6,6-tetramethyl piperidine (TMP). The photosensitizer we used was either a hydrophobic dye, tetraphenylporphyrin (TPP), or a cationic dye, methylene blue (MB). We incorporated the photosensitizers in the bilayer membranes or the aqueous inner compartments of one set of vesicles and solubilized the substrate molecules in the bilayer regions of another set of vesicles. These two sets of vesicle dispersions were then mixed to give the irradiation samples. By isolating the products in photooxidation of the olefins, and by detecting the electron spin resonance (ESR) spectrum of the stable nitroxide radical in the reaction of $^1O_2$ and the amine, we demonstrated that singlet oxygen produced in the bilayer region or in the inner water pool of a vesicle was capable of diffusing out of the vesicle and reacting with substrates which were located in other vesicles. Furthermore, the olefin oxidation products were quite different from those observed with the same substrates in homogeneous solutions.

Results and Discussion

General. The vesicles selected for investigation were prepared by sonicating the equimolar mixture of a cationic surfactant (octyltrimethylammonium bromide, 8.2×10^{-2} M) and an anionic surfactant (sodium laurate, 8.2×10^{-2} M) in buffered solution (pH = 9.2) for 30 min at 50 °C.[10] Formation of vesicles from the mixture of these cationic and anionic surfactants arises from the strong electrostatic interaction between the oppositely charged headgroups of the components. As a result, the mean effective headgroup area decreases considerably, while the mean hydrophobic volume of the tails remains the same. Thus, this dynamic ion pairing yields a pseudo-double-tailed zwitterionic surfactant, which is known to have the preferred geometry of a vesicle-forming surfactant.[10a] We demonstrated the vesicle formation by transmission electron microscopy with negative technique (stained with uranyl acetate). The unilamellar layer of the vesicle was clearly shown in the electron micrograph, and its thickness was measured to be ~4−5 nm. The vesicles were polydisperse with radii ranging from ~80 to 150 nm, and the average vesicle radius was ~100 nm. Vesicles formed in this way were stable, and the solution was optically clear.[10a] The substrates DMOS, TS, DPB and TMP can all be easily incorporated into the bilayer membranes of the vesicles by sonication, since they are hydrophobic. Generally, the concentration of the olefins was $\sim 2.0 \times 10^{-3}$ M, and that of TMP was $\sim 2.0 \times 10^{-2}$ M, corresponding to thousands of substrate molecules in each vesicle (see below). The sensitizer TPP can be solubilized in the bilayer membranes, while MB can be encapsulated in the aqueous inner compartments of the vesicles. The concentration of the sensitizer was generally $\sim 1.0 \times 10^{-4}$ M.

In the case of TPP as the sensitizer, the vesicles only incorporating the substrate and those only solubilizing the sensitizer were prepared separately as described above. Equal volumes of the two samples were then mixed. Although sonication was carried out during preparation of the component solutions, the final mixture was not sonicated. In this way intermixing of solubilizates was prevented. In the case of MB as the sensitizer, we encapsulated this water-soluble dye in the inner compartments of one set of vesicles and solubilized the substrates in the bilayers of another set of vesicles. The MB-containing vesicles were prepared as described above with the substitution of saturated MB solution for water. Vesicles formed in the presence of MB were equal in size to those formed in pure water. These MB-containing vesicles were chromatographed through a Sephadex G-25 column to remove the MB from the exterior of the vesicles,[11] then the eluting vesicle dispersions were diluted to 1.0×10^{-4} M. Mixing of the substrate- and the MB-containing vesicles gave the irradiation samples. Irradiation of the samples saturated with oxygen by bubbling the gas was carried out by using a 450-W Hanovia Hg lamp as the light source, and a glass filter was used to cut off the light with a wavelength below 400 nm, ensuring the absence of direct excitation of the alkene and the amine substrates. For the alkene samples, after irradiation the products were extracted with ether and analyzed by GC. Generally, material balance was greater than 95%. For TMP samples, the nitroxide radical (a product of reaction of $^1O_2$ with TMP)[12] was analyzed by ESR spectroscopy. The yield of this radical was measured on the basis of the intensity of the ESR signal by comparison with that of a calibrated concentration of the radical in solution.

The above photosensitized oxidations for the samples in which water was replaced by D_2O as dispersion medium were also performed. The products for all the substrates were identical to those in the vesicles in water dispersions. However, the efficiencies for product formation were significantly enhanced.

Reaction of Singlet Oxygen Generated in the Bilayer Regions of One Set of Vesicles with Olefins Located in Another Set of Vesicles. As mentioned above, in the case of TPP as the sensitizer, the vesicles only incorporating the substrate and those only solubilizing the sensitizer were prepared separately and then mixed to give the irradiation samples.

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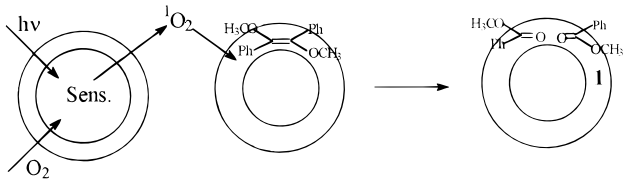
Table 1. Quantum Yields of the Product Formation in Photosensitized Oxidation of Olefins in Vesicles

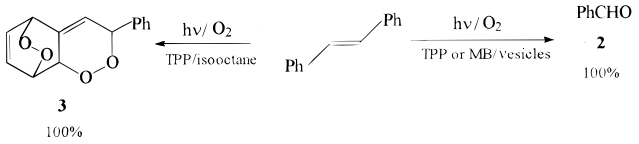
sens.	TPP						MB					
sub.	DMOS		TS		DPB		DMOS		TS		DPB	
medium	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O	H_2O	D_2O
quantum yield (%)[a]	11	44	0.70	6.3	0.40	1.5	3.8	6.9	–	–	–	–

[a] Error limit is ~2%.

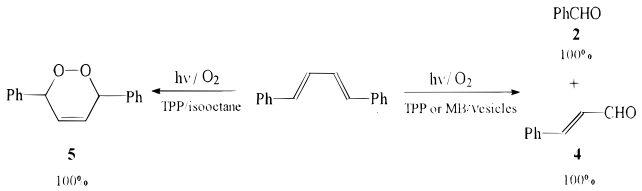
Scheme 1



Scheme 2



Scheme 3



Irradiation of the samples saturated with oxygen resulted in oxidation of the olefins. For all three olefins, only the products derived via 1,2-cycloaddition of singlet oxygen to the alkenes were detected (Schemes 1−3). These results were in contrast with those of TPP-photosensitized oxidation of DPB and TS in homogeneous solutions where the main products were derived from 1,4-cycloaddition of $^1O_2$ to the dienes (Schemes 2−3).

A control experiment was carried out: the mixed solution prepared from sensitizer-containing vesicles and substrate-containing vesicles was stored in the dark at room temperature for 1 day and then was irradiated as described above. The efficiency of the product formation for the photosensitized oxidation was found to be identical within experimental error limit to that of the sample which was immediately irradiated after the preparation. This observation suggests that the intervesicular exchange both of the substrate and the sensitizer did not occur and that the photosensitized oxidation process involved the generation of $^1O_2$ in one vesicle and reaction with alkene molecules in the other vesicles.

The vesicles used in this study have an aggregation number (number of surfactant molecules per vesicle) in the region of 10^5-10^6, and the average aggregation number is $\sim 7.2 \times 10^5$, as estimated from the vesicle size and the volume of the surfactant molecule. Thus, at surfactant concentration of 8.2×10^{-2} M the vesicle population is equivalent to a molarity of $\sim 1.1 \times 10^{-7}$ M, which in turn gives the intervesicular distance on average to be ~134 nm.[13] On the other hand, the species $^1O_2$ is small and uncharged and has a relatively long lifetime and properties which allow it to diffuse a long distance in nonviscosity media. The average diffusion length of $^1O_2$ molecule in aqueous solution is estimated to be ~780 nm, and even longer in D_2O (2500 nm).[14] This diffusion length is much larger than the intervesicular distance estimated above, and the $^1O_2$ generated in one vesicle is indeed capable of diffusing into other vesicles to react with the alkene molecules.

The efficiencies for the product formation of the above photosensitized oxidation were measured. Table 1 gives the quantum yields of the product formation for the photosensitized oxidation. Evidently, the efficiencies of photosensitized oxidation of DPB and TS were lower compared with that of DMOS. These lower efficiencies are probably due to the quantum yields of their reactions with $^1O_2$ that are smaller than that of DMOS. Significantly, the quantum yields of the product formation for all of the samples in D_2O medium were greater than those in H_2O. The quantum yields increased 4−9 times when the solvent was changed from H_2O to D_2O. This observation strongly supports the singlet oxygen mechanism for the oxidation, since it has been established that the lifetime of $^1O_2$ is 10 times longer in D_2O than in H_2O.[15]

The process of the above photosensitized oxidation involves the following three steps: (1) generation of $^1O_2$ by energy transfer from the triplet state of the sensitizer to the ground state of O_2, (2) diffusing of $^1O_2$ from the generation locus to the reaction site, and (3) reaction of $^1O_2$ with the target molecule. The quantum yield (Φ) of the product formation should be a product of the quantum yield for the formation of $^1O_2$ (ϕ_p), the fraction of the generated $^1O_2$ diffusing to the reaction sites (ϕ_d), and the efficiency of the reaction between $^1O_2$ and the target molecules (ϕ_q).

$$\Phi = \phi_p \cdot \phi_d \cdot \phi_q \qquad (1)$$

The quantum yield (ϕ_p) for $^1O_2$ production photosensitized by TPP in hexane was reported to be 0.58.[16] We proposed that the environment in the bilayer of the vesicle is nonpolar and might be similar to that of hexane; thus, the ϕ_p value for TPP in vesicles was suggested to be 0.58. We take DMOS as the representative of the olefins. The efficiency (ϕ_q) for the bimolecular reaction between $^1O_2$ and DMOS can be calculated by eq 2,

$$\phi_q = k_r \cdot [Q]/(k_r \cdot [Q] + 1/\tau) \qquad (2)$$

where k_r is the bimolecular reaction constant between $^1O_2$ and DMOS, and has the value of 1.58×10^7 $M^{-1} \cdot s^{-1}$ in nonpolar solvents,[17] τ is the $^1O_2$ lifetime and has the value of ~30 μs in hexane.[18] [Q] is the local concentration of DMOS in vesicles. As mentioned above, the concentration of the olefin in the dispersion we used was $\sim 2.0 \times 10^{-3}$ M, and the vesicle population was equivalent to a molarity of $\sim 1.0 \times 10^{-7}$ M.

(13) In a 1.1×10^{-7} M solution, the average distance between the solutes is ~134 nm, see: Turro, N. J. *Modern Molecular Photochemistry*; Benjamin/Cummings Pub. Co., Inc.; California, 1978; p 319.

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Thus, on average each vesicle contains $\sim 2.0 \times 10^4$ molecules of the olefin. The volume of each vesicle was calculated to be 4.8×10^{-19} l from the radius (100 nm on average) and thickness (4 nm on average) of the vesicle. As a result, the local concentration [Q] of DMOS in vesicles was 6.9×10^{-2} M. Thus, ϕ_q was calculated from eq 2 to be 0.96, suggesting that almost all of the $^1O_2$ diffusing into the olefin-bound vesicles reacted with the substrate molecules. By using the above ϕ_p and ϕ_q data and the values of Φ for DMOS in Table 1, we obtained ϕ_d value in H_2O medium from eq 1 to be $\sim$20% and that in D_2O dispersion, $\sim$80%. Evidently, a significant fraction of $^1O_2$ generated in the bilayer of one vesicle diffused out of the vesicle and entered into the other vesicles.

It is worth noting that the products of the photosensitized oxidation of TS and DPB in vesicles are remarkably different from those in homogeneous solutions (Schemes 2 and 3). For example, irradiation of DPB solution in isooctane yielded endoperoxide (**5**), a 1,4-cycloaddition product of the diene to $^1O_2$ as the unique product (Scheme 3).[19] In sharp contrast, the photosensitized oxidation of DPB in vesicles produced cinnamaldehyde (**4**) and benzaldehyde (**2**) in quantitative yield as described above. Evidently, these products were derived from an intermediate dioxetane, a 1,2-cycloaddition product.[19] Similarly, while in isooctane solution the photosensitized oxidation of TS gave the 1,4-cycloaddition product, diendoperoxide (**3**),[19] that in vesicles yielded exclusively the 1,2-cycloaddition product, benzaldehyde (**2**) (Scheme 2). Control experiments demonstrated that the 1,4-cycloaddition products **3** and **5** in vesicle dispersions were stable under our experimental conditions, suggesting that these products were indeed not produced in the photooxidation of olefins in vesicle samples. The preferential formation of the products of 1,2-cycloaddition over those of 1,4-cycloaddition in vesicles is probably best explained in terms of a greater difficulty in achieving the necessary geometry for 1,4-cycloaddition in this organized medium. It has been established that *trans,trans*-1,4-diphenyl-1,3-butadiene in solution exists in two conformational isomers: cisoid and transoid.[20] At equilibrium the main conformer is the transoid ($\sim$99%), and the cisoid is presented only in $\sim$1%. The 1,4-cycloaddition of singlet oxygen to 1,3-diene to form endoperoxide is concerted and analogous to the Diels−Alder reaction.[19a] This reaction requires a six-membered ring transition state. Only the cisoid conformer can satisfy such a requirement, and in order to undergo 1,4-cycloaddition with singlet oxygen the transoid first has to be isomerized to the cisoid. Due to the kinetic equilibrium between the two conformers in solution, the cycloaddition can proceed until all of the diene is converted to the products. Obviously, in vesicles the organized semirigid environment prevents DPB from the conformational change. Thus, only the 1,2-cycloaddition products were obtained.

Reaction of Singlet Oxygen Generated in the Inner Water Pools of One Set of Vesicles with Olefins Located in Another Set of Vesicles. In the case of MB-sensitization, the sensitizer was encapsulated in the inner water pools of vesicles. The concentration of MB we used was $\sim 1.0 \times 10^{-4}$ M, and the number of MB molecules in each water pool of the vesicle was calculated to be $\sim$870 from the population of the vesicles. The samples were prepared by mixing the MB-and substrate-containing vesicles. Irradiation of the oxygen-saturated samples with $\lambda > 400$ nm light resulted in the oxidation of the olefins. This observation illustrates that singlet oxygen generated in the intravesicular water pool can diffuse across the bilayer membrane of the vesicle and enter into the bilayer of another vesicle to react with the target molecules. The products for all three olefins were identical to those for TPP-sensitization. Again, among the products only the 1,2-cycloaddition products of $^1O_2$ to the olefins were detected. The quantum yields for product formation both in H_2O and in D_2O media are given in Table 1. As in the case of TPP-sensitization, the quantum yield of product formation for DMOS was greater in D_2O dispersion than that in H_2O medium, suggesting that the singlet oxygen mechanism operated. It was found that MB underwent autoxidation during the irradiation under our experimental conditions.[21] Thus, the irradiation duration for these samples was generally less than 1 h. Under such irradiation duration the conversions of the starting materials for TS and DPB were less than 2%. GC chromatograph showed that the products were definitely produced, but the amounts of the products were too small to be accurately measured. Thus, we could not obtain the accurate quantum yields of product formation for TS and DPB. Table 1 revealed that the quantum yields of the photosensitized oxidation for the olefins in MB-sensitization were significantly smaller than those in TPP-sensitization. This reduction in quantum yields for product formation evidently arose from the smaller quantum yield of $^1O_2$ production (ϕ_p) and the smaller fraction of the generated $^1O_2$ diffusing to the reaction sites (ϕ_d) in the case of MB-sensitization compared with those of TPP-sensitization. The quantum yield of $^1O_2$ production for MB in TX-100 micellar solution has been reported to be 0.47,[22] while that for TPP in hexane is 0.58.[16] By using the ϕ_q value mentioned in the above section (96%) and the data of Φ for DMOS in Table 1, ϕ_d was calculated from eq 1 to be $\sim$8% in H_2O medium and $\sim$15% in D_2O dispersion. Indeed these values are smaller compared with those in TPP-sensitization.

Reaction of Singlet Oxygen with 2,2,6,6-Tetramethylpiperidine in Vesicles. The evidence for the capability of $^1O_2$ diffusing from the sensitizer-bound vesicle to the substrate-bound vesicles based on the photosensitized oxidation of olefins is further strengthened by ESR measurements. It has been well established that reaction of sterically hindered amines with $^1O_2$ yields stable free radicals, nitroxides, which can be easily detected by ESR spectroscopy.[12] Thus, we incorporated 2,2,6,6-tetramethylpiperdine (TMP) in the bilayers of one set of vesicles, solubilized the photosensitizers in the bilayers or the aqueous inner compartments of another set of vesicles, and then mixed the two sets of vesicle dispersions to prepare the irradiation samples. The photosensitized oxidation of the amine was examined by ESR spectroscopy. Figure 1 shows the ESR spectrum for the sample prepared from TPP- and TMP-bound vesicles in H_2O medium. This spectrum was obtained after 10 min irradiation of the oxygen-saturated sample and characterizes nitroxide free radical. The line width, the *g*-factor, and the nitrogen splitting of the radical are identical to those reported in the literatures[12] within experimental error limits. Control experiments revealed that light, sensitizer, oxygen, and TMP all are essential for production of the ESR signal. Evidently, the singlet oxygen generated in one vesicle can diffuse out and enter into other vesicles to react with TMP and yield the nitroxide radical. The amount of the generated radical was determined by comparison with the nitroxide solutions of known concentrations. Figure 2 gives the plot of the concentration of

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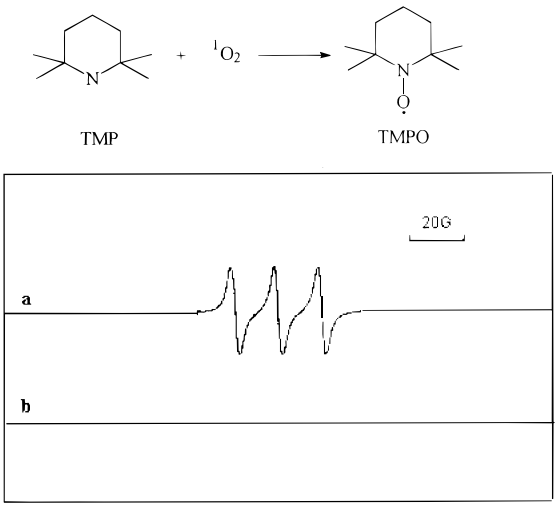

Figure 1. ESR spectrum of nitroxide radical generated by irradiation of the oxygen-saturated sample prepared from the mixture of TPP- and TMP-bound vesicles. Irradiation time: (a) 10 min, (b) 0 min.

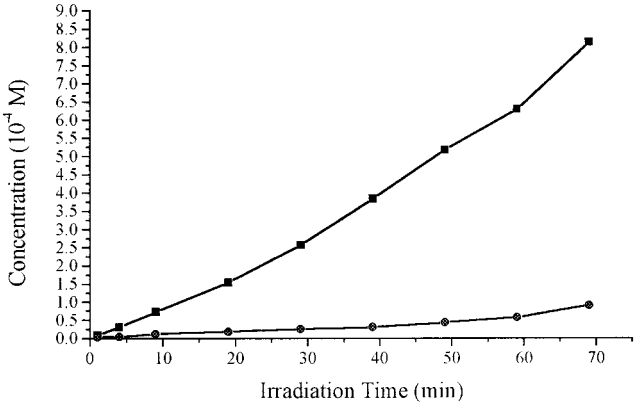

Figure 2. Plots of nitroxide concentration as a function of the irradiation time. ●, TPP in H_2O; ■, TPP in D_2O.

Table 2. Rates and Quantum Yields of Nitroxide Radical Formation in the Photosensitized Oxidation of 2,2,6,6-Tetramethylpiperidine in Vesicles

sen.	medium	rate of radical formation ($\times 10^6$ M·min^{-1})	quantum yields of radical formation[a] (%)
TPP	H_2O	2.8	10.2
	D_2O	13	47.9
MB	H_2O	1.3	3.7
	D_2O	5.7	16.4

[a] Error limit is ~2%.

the generated nitroxide radical as a function of the irradiation time. The slope of the plot represents the rate of the radical production, which in turn gives the quantum yield of the radical formation (Table 2) since the incident light intensity we used is known. The experiments in D_2O dispersions were also performed, and the results are presented in Figure 2 and Table 2. The quantum yield of the nitroxide radical formation in D_2O is ~5 times greater than that in H_2O medium, suggesting that singlet oxygen is indeed involved.

For testing the capability of $^1O_2$ diffusion from the inner water pool of one vesicle to the bilayer of another vesicle, the samples were prepared from the MB- and TMP-bound vesicles by the method described above. Irradiation of the oxygen-saturated samples indeed resulted in the nitroxide radical as evidenced by the ESR spectrum. Figure 3 gives the plots of the concentration of the generated nitroxide as a function of irradiation time,

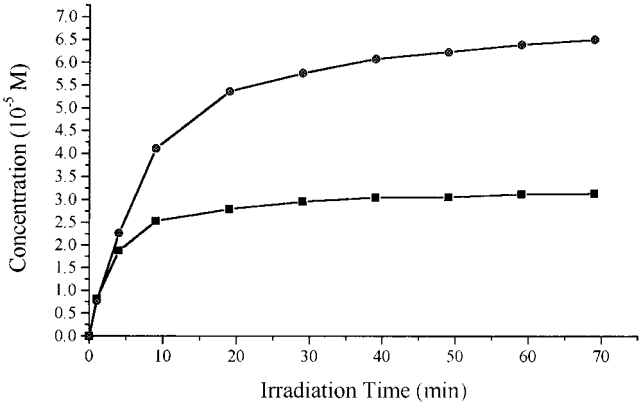

Figure 3. Plots of nitroxide concentration as a function of the irradiation time. ●, MB in D_2O; ■, MB in H_2O.

and the rates and quantum yields of the radical formation both in H_2O and D_2O dispersions are shown in Table 2. The efficiency of the nitroxide radical formation increased ~ 4 times when the medium was changed from H_2O to D_2O. This effect of D_2O is compared with those in the photosensitized oxidation of olefins. Both in H_2O and D_2O dispersions MB was less efficient than TPP in sensitizing the photooxidation of TMP, due to the smaller quantum yield of $^1O_2$ production as mentioned above and the smaller fraction of $^1O_2$ diffusing from the inner water pool of one vesicle into other vesicles. Furthermore, rapid autoxidation of MB is clearly shown as in the case of the photosensitized oxidation of olefins. The plateau of the plot for MB sensitizer in Figure 3 indicates that after 30 min irradiation the total amount of MB was autoxidized.

The quenching constant of singlet oxygen by TMP (k_r) has been reported to be ca. 8×10^8 M^{-1}·s^{1-} in nonpolar solvents.[12c] The local concentration of TMP in vesicles was calculated to be 0.3 M based on the concentration of TMP in bulk solution and the population of the vesicles. Assuming that the lifetime of $^1O_2$ in nonpolar solvents is 30 μs, we could calculate the efficiency of the reaction of $^1O_2$ with TMP (ϕ_q) in the above vesicle system from eq 2. This value of ϕ_q was found to be close to 100%. By using the data of the quantum yields of the nitroxide radical formation (Φ) in Table 2 and the quantum yields of $^1O_2$ production (ϕ_p) with the two sensitizers (see the above section), the fractions (ϕ_d) of $^1O_2$ diffusing from the generation locus into the TMP-bound vesicles were calculated from eq 1 to be 18% in H_2O and 82% in D_2O for TPP, and those were 8% in H_2O and 35% in D_2O for MB-sensitization. These values are consistent with those obtained in the experiments of the photooxidation of olefins.

Conclusions

The studies of the photosensitized oxidations of the olefins and the amine provide unambiguous evidence that singlet oxygen, when produced in the bilayer regions or the inner water pools of one set of vesicles, is capable of diffusing out of the vesicles, crossing the dispersion medium, and entering into the bilayers of other vesicles where it reacts with the target molecules. The quantum yield of the product formation in such photosensitized oxidation is dependent on the efficiency of $^1O_2$ production, the fraction of $^1O_2$ diffusing from the generation locus to the reaction sites, and the efficiency of the reaction of $^1O_2$ with the substrates. Under our experimental conditions, ~8% of the $^1O_2$ generated in the inner aqueous compartments of vesicles in H_2O can diffuse into other vesicles and does ~15% of the $^1O_2$ in D_2O dispersions. For the $^1O_2$ produced in

the bilayers of vesicles, an even greater fraction (~20% in H_2O, and ~80% in D_2O) can enter into the bilayers of other vesicles. Since the local concentration of the substrates in the bilayers of the vesicles was large enough, the singlet oxygen diffusing into the vesicle bilayers was deactivated mainly via the reaction with the substrates. The photosensitized oxidation of DPB and TS in vesicles resulted exclusively in the 1,2-cycloaddition products, which is contrast with those in homogeneous solutions where the 1,4-cycloaddition products of the diene to $^1O_2$ were the unique products. This observation suggests that the organized semi-rigid environment in vesicles prevents the diene from the conformation change to adapt the necessary geometry for 1,4-cycloaddition.

Experimental Section

Materials and Instrumentation. TS, DPB, TMP, TPP, and MB were purchased from Fluka and used as received. DMOS was synthesized according to a literature procedure.[23] Surfactants octyltrimethylammonium bromide and sodium laurate were Aldrich products, and were recrystallized twice from ethanol−ether before use. Doubly distilled water was used throughout this work. D_2O was purchased from Sigma. Gas chromatography was performed on a Shimadzu GC-7A with a 3% OV-17 column. Mass spectra were run on a VGZAB GC−MS spectrometer. ^1H NMR spectra were recorded at 400 Hz with a varian VXR-400 spectrometer. ESR spectra were measured on a Bruker ESP-300E spectrometer. UV absorption spectra were recorded on a UV-1601 (PC) S spectrometer. Electron microscopy was examined on a Hitach H-600 electron microscope.

Preparation of Samples. To the equimolar mixture of octyltrimethyl ammonium bromide and sodium laurate was added H_2O or D_2O buffer solution (pH = 9.2, $Na_2B_2O_7$) to form a suspension solution with the concentration of $\sim 8.2 \times 10^{-2}$ M for each surfactant. A known amount of substrate was added to the above suspension. The concentration of the substrate in the suspension was $\sim 2.0 \times 10^{-3}$ M for olefins, and $\sim 2.0 \times 10^{-2}$ M for TMP. The suspension was sonicated for 30 min at 50 °C. The solution became clear, and the substrate-containing vesicles were produced. By using the same procedure the TPP-containing vesicle dispersions were prepared. The concentration of this sensitizer was $\sim 1.0 \times 10^{-4}$ M. Mixing of equal volumes of the above substrate- and sensitizer-containing vesicle dispersions gave the irradiation samples.

MB-containing vesicle dispersions were prepared from aqueous saturated MB solution by the procedure described above. The vesicles were chromatographed through a Sephadex G-25 column to remove the MB from the exterior of the vesicles. The volume of the eluting dispersion was adjusted by addition of H_2O to make the bulk concentration of MB to be $\sim 1.0 \times 10^{-4}$ M. The irradiation samples were prepared by mixing equal volumes of the MB- and substrate-containing dispersions.

Negative Staining Electron Microscopy. The vesicle samples were negatively stained with a 2% (w/w) uranyl acetate solution and examined on a Hitach H-600 electron microscope.

Photooxidation of Olefins and Product Analysis. The sample in a Pyrex reactor was bubbled with oxygen during irradiation. A 450-W medium-pressure Hanovia Hg lamp was employed as the light source, and a glass filter was used to cut off light with a wavelength below 400 nm. The filter thus ensured the absence of direct excitation of the olefin substrates. After irradiation, the products were extracted with ether, analyzed by GC, and identified by their spectral properties and by comparison with authentic samples.

ESR spectra. The ESR spectra were registered by a Bruker ESP-300E spectrometer operating in the X band at room temperature. The microwave frequency was 9.83 GHz and the power was 5.05 mW; the modulation amplitude was 2.035 G. The oxygen-saturated sample in a flat quartz cell was irradiated directly in the cavity of the ESR spectrometer by the light source as described in the above section.

Acknowledgment. We thank the National Science Foundation of China, and the Bureau for Basic Research, Chinese Academy of Sciences for financial support. We also thank Professor Richard G. Weiss of Georgetown University for his encouraging discussion.

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