Effect of Micelles on Oxygen-Quenching Processes of Triplet-State Para-Substituted Tetraphenylporphyrin Photosensitizers

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Development of clinically valuable porphyrin drugs for use in photodynamic therapy requires characterization of membrane effects on porphyrin excited-state dynamics and bimolecular quenching with oxygen. This study reports on the quenching of triplet-state tetraphenylporphyrin (TPP), 5-(*p*-carboxyphenyl)-10,15,20-triphenylporphyrin ((*p*-COO^-)$_1$TPPH_2), 5-(*p*-aminophenyl)-10,15,20-triphenylporphyrin ((*p*-NH_2)$_1$TPPH_2), and 5-(*p*-hydroxyphenyl)-10,15,20-triphenylporphyrin ((*p*-OH)$_1$TPPH_2), by dissolved oxygen in a range of environments, such as cyclohexane solution and aqueous micellar solutions of tetradecyltrimethylammonium bromide (TTAB) and poly(ethyleneglycol)-*p*−*tert*-octylphenol (TX-100). The bimolecular quenching rate constants were found to be similar for all environments and porphyrins studied here, but the observed rate constants in aerated solutions varied with solvent and substituent types. These results indicate that location of porphyrins within the micelles was affected by the nature of the detergent headgroup as well as by the porphyrin substituent. They also support the conclusion that an oxygen concentration gradient exists in the micelles with the highest oxygen concentration at the center.

Introduction

Porphyrins and their derivatives have been known to localize preferentially in tumors and have been, therefore, studied with great interest for cancer therapy as well as sensitizers for photodynamic therapy (PDT).[1−12] Despite their current applications in medicine and pharmacology, there remain several critical issues that need to be further understood concerning sensitizer delivery and uptake mechanisms into cells, localization within cells, and cellular targets.[13−15] Also critical are the photophysics of the sensitizer and the type molecular mechanism involved in the cellular necrosis or apoptosis.[7,12,16,17] To address these issues, researchers have used model membrane systems and have incorporated porphyrins into micelles and lipid bilayers.[18−30] The results of the studies show that porphyrins can be solubilized and monodispersed in micelles, but it is debated where the porphyrin is located, if and how it is oriented, and how the structural characteristics of the porphyrin correlate with its location and orientation.[23,24,27,29−32]

To address questions concerning how membrane incorporation depends on the structural and electronic properties of porphyrins and to determine what factors affect porphyrin−micelle interactions, Vermathen et. al. performed a systematic study with porphyrins in micelles using NMR and UV−visible spectroscopy. They synthesized four water insoluble tetraphenylporphryin (TPP) derivatives (see Figure 1 for a partial list) and allowed them to freely incorporate into micelles of cationic tetradecyltrimethylammonium bromide (TTAB), anionic sodium dodecyl sulfate (SDS), and nonionic poly(ethyleneglycol)-*p*−*tert*-octylphenol (TX-100) surfactants. The results reveal that not all porphyrins studied freely diffused into micelles, and the ability of the porphyrin to localize in the micelles depends on the nature on the substituent, the surfactant headgroup, and the pH of the solution.[26] This study examined structure−solubility relationships, which are a critical in PDT for sensitizer activity and efficacy, but the photophysics of the sensitizers under these conditions remain to be investigated.

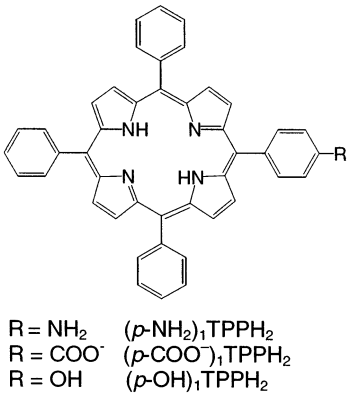

Figure 1. Schematic representation of the structures of the mono *p*-phenyl-substituted tetraphenylporphyrins.

One of the proposed mechanisms for PDT tumor destruction is energy transfer from the excited sensitizer triplet state to the ground state of oxygen, creating singlet oxygen, which is thought to be responsible for tumor necrosis.[12,33−35] Because the photosensitizing efficiency of PDT agents is strictly correlated with the properties of their excited states,[16] and the interaction of a photosensitizer with biological membranes can alter its photodynamic action and ability to induce necrosis of tumors, it is essential to understand the fundamentals of how membrane environments affect sensitizer photophysics.[12,16,36,37]

To this end, we studied the excited-state properties of sensitizers under similar conditions used in the study by

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Vermathen et. al. using UV−visible, fluorescence, and transient absorption spectroscopy.[26] The triplet-state lifetimes and rates of oxygen quenching in aerated solutions of three monosubstituted, water insoluble porphyrins (see Figure 1), 5-(*p*-carboxyphenyl)-10,15,20-triphenylporphyrin ((*p*-COO^-)$_1$TPPH$_2$), 5-(*p*-aminophenyl)-10,15,20-triphenylporphyrin ((*p*-NH_2)$_1$TPPH$_2$), and 5-(*p*-hydroxyphenyl)-10,15,20-triphenylporphyrin ((*p*-OH)$_1$TPPH$_2$) and unsubstituted tetraphenylporphyrin (TPP) incorporated in TTAB and TX-100 and in cyclohexane were measured and compared.

Our results show that the incorporation of the various porphyrins into TTAB and TX-100 micelles has the effect of lengthening the observed triplet lifetimes in aerated solution as compared to aerated cyclohexane. The bimolecular quenching rate constant is unaffected by the presence of micelles, but the observed rate constant varies with substituent type and micelle type. Variation in the observed rate constants in aerated solutions are attributed to varying oxygen solubilities within the micelles and photosensitizer location in the micelle interior or a combination of location and higher viscosity in the micelle interior as compared with cyclohexane.[38] These results suggest that interactions of photosensitizers with solvent and membranes have significant effects on the singlet oxygen production and therefore, possibly, on tumor destruction in PDT.

Experimental Section

Sample Preparation. Tetraphenylporphyrin was purchased from Aldrich. Water insoluble (*p*-COO^-)$_1$TPPH$_2$, (*p*-NH_2)$_1$-TPPH$_2$, and (*p*-OH)$_1$TPPH$_2$ were synthesized using the Adler mixed aldehyde approach.[26] Their structures are shown in Figure 1. After purification by column chromatography on a silica gel and elution with CH_2Cl_2, (p-COO^-)$_1$TPPH$_2$, (p-NH_2)$_1$TPPH$_2$, and (p-OH $_1$TPPH$_2$) were allowed to freely diffuse into aqueous nonionic micellar solutions of 2.3 mM TX-100 (critical micelle concentration 0.24 mM at 298 K)[39] or into 16 mM aqueous cationic micellar solutions of TTAB (critical micelle concentration 3.8 mM at 298 K)[40] both at neutral pH. Micelle solutions of TPP were prepared by mixing solutions of dissolved surfactant and TPP in chloroform with methanol, evaporating off the solvent and then redissolving with appropriate amounts of milli-Q pure water. Porphyrin concentrations were adjusted such that maximum optical density in a 2 mm cell was not above 1.0 and final concentrations ranged from 3 to 6 μM. Aerobic samples were allowed to equilibrate with atmospheric oxygen. For anaerobic experiments, deoxygenated solutions were prepared by bubbling with purified argon gas while stirring for 1 h. The resulting samples were kept under argon during experiments. Our argonation procedure most likely did not exclude oxygen completely and hence lifetimes observed under these conditions may still be quenched by residual oxygen.

Instrumentation. Ground-state absorption spectra were recorded on an HP-8452A UV−visible spectrophotometer with 2 nm resolution. Static emission spectra were measured with a Perkin-Elmer LS-50B fluorometer. Transient absorption difference spectroscopy on the nanosecond through microsecond time scale was performed using a pump−probe approach that has been described previously.[41] Briefly, the excitation source was a Quanta Ray DCR-2 Nd:YAG laser that produced 7 ns (fwhm) pulses of 355 nm light (0.07−0.08 mJ/mm^2). The probe beam consisted of white light from a microsecond short arc flash lamp. Time resolution was provided by gating the intensifier of a PAR 1420 intensified diode array detector for 20 ns. Delaying the intensifier gate pulse relative to the 7 ns laser pulse controlled the time at which a measurement was made. By delaying the probe flash and the intensifier gate pulse in tandem, spectra at delay times from nanoseconds to seconds could be acquired. After sample excitation at 355 nm, the absorption difference spectra were recorded at 6 delay times of 30 ns, 100 ns, 500 ns, 1 μs, 3 μs, and 10 μs. In experiments with micellar solutions fresh sample was pumped into the optical path after each excitation pulse. Stirring was used in cyclohexane experiments because the flow system was incompatible with organic solvents.

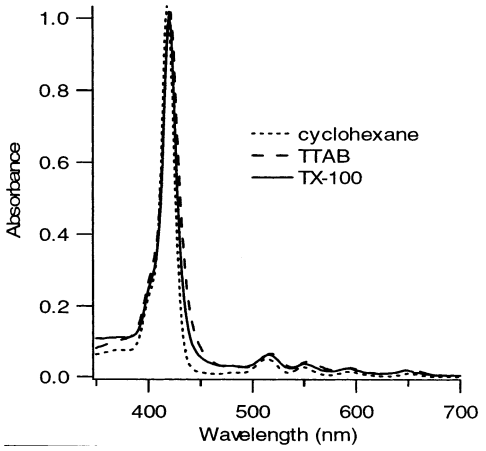

Figure 2. Ground-state electronic absorption spectra of (*p*-NH_2)$_1$TPPH$_2$ in cyclohexane, TX-100, and TTAB, with a 1 cm path length.

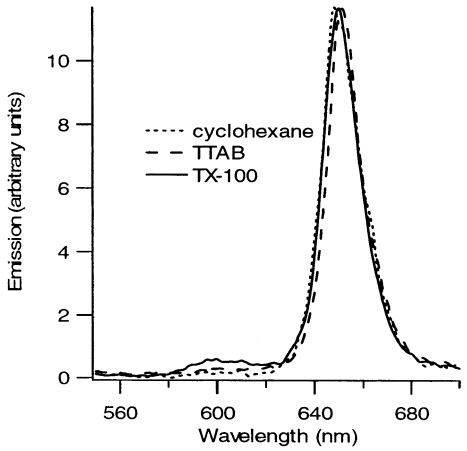

Figure 3. Normalized emission spectra of (*p*-COO^-)$_1$TPPH$_2$ in cyclohexane, TX-100, and TTAB with a 1 cm path length.

Results

Static Spectral Measurements. The ground-state electronic absorption spectra and static fluorescence spectra of TPP, (*p*-COO^-)$_1$TPPH$_2$, (*p*-NH_2)$_1$TPPH$_2$, and (*p*-OH)$_1$TPPH$_2$ in cyclohexane, aqueous TTAB, and aqueous TX-100 solution were similar to those of (*p*-NH_2)$_1$TPPH$_2$, which are shown in Figures 2 and 3. For (*p*-COO^-)$_1$TPPH$_2$ and (*p*-NH_2)$_1$TPPH$_2$ there is an intense absorption peak in the Soret region at 418 nm in cyclohexane, 420 nm in TX-100, and 420 nm in TTAB, in addition to the four weaker peaks corresponding to the Q-bands absorbing at 512−650 nm. Similar absorption profiles were seen for TPP, with the Soret band absorbing at 416 nm in cyclohexane, 418 nm in TX-100, and 418 nm in TTAB and Q-bands absorbing from 514 to 644 nm. For (*p*-OH)$_1$TPPH$_2$ in TX-100 there is an intense Soret band at 420 nm and in TTAB at 418 nm with Q-bands from 550 to 650 nm. The similarity in the absorption profiles of the porphyrins in cyclohexane and in micelles indicates that the porphyrins are monodispersed and remain dissolved in nonpolar environments.

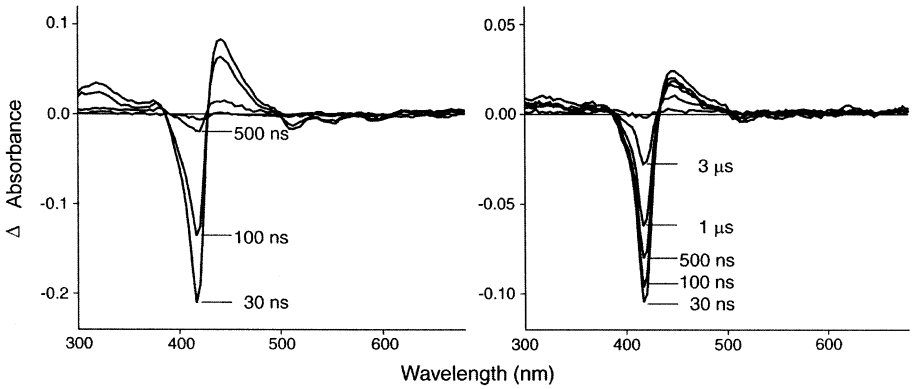

Figure 4. Transient absorption spectra of aerated $(p\text{-NH}_2)_1\text{TPPH}_2$ in aqueous TX-100 (left) and cyclohexane (right), excited at 355 nm

TABLE 1: Fitting Parameters and Quenching Constants for $(p\text{-NH}_2)_1\text{TPPH}_2$, $(p\text{-COO}^-)_1\text{TPPH}_2$, $(p\text{-OH})_1\text{TPPH}_2$, and TPP in Various Solvents

sample	solvent	τ_2 (μs)	k_q ($M^{-1}s^{-1}$)
$(p\text{-NH}_2)_1\text{TPPH}_2$	cyclohexane	0.210	2.1×10^9
	TX-100	2.1	6.2×10^8
	TTAB	1.6	8.1×10^8
$(p\text{-COO}^-)_1\text{TPPH}_2$	cyclohexane	0.235	1.9×10^9
	TX-100	2.2	5.9×10^8
	TTAB	1.9	6.8×10^8
$(p\text{-OH})_1\text{TPPH}_2$	TX-100	2.2	5.9×10^8
	TTAB	1.7	7.6×10^8
TPP	cyclohexane	0.210	2.1×10^9
	TX-100	1.7	7.6×10^8
	TTAB	0.86	1.5×10^9

There is a characteristic emission band near 650 nm corresponding to S_1 to ground-state emission[42] for the four porphyrins in all solvents. There were observed differences in emission intensities in the various solvents, which may be attributed to solvent effects and/or porphyrin location within the micelles.[43,44] Typical fluorescence quenching and wavelength shifts, which have been observed for aggregates[44,45] were not observed for the four porphyrins studied. In agreement with the conclusion drawn from the absorption profiles, the emission spectra suggest that the four porphyrins exist as monomeric species in a nonpolar environment.

Transient Absorption Difference Spectra. Figure 4 shows the transient absorption difference spectra of aerobic $(p\text{-NH}_2)_1\text{TPPH}_2$ in TX-100 and cyclohexane following excitation at 355 nm. In all these difference spectra there is one negative peak near 420 nm corresponding to a bleach signal from the Soret band. There are transient absorption bands from 320 to 390 nm and near 460 nm that are attributed to triplet-state absorption. Tetrapyrrolic excited singlet-state lifetimes are usually less than 20 ns and have mostly decayed to the ground state or to the triplet state via intersystem crossing by the time the system is first probed, at 30 ns.[42,46−52] Therefore, excited singlet-state contributions to the transient absorption signals are negligible. The observed excited triplet-state lifetimes and the quenching rate constants for the bimolecular reaction with oxygen for each sample are summarized in Table 1. Lifetimes of porphyrins in aerated solutions of nonionic TX-100 micelles were longer than those seen in cyclohexane or TTAB and were independent of substituent. Lifetimes of the porphyrins in aerated TTAB were found to be substituent dependent, with $(p\text{-OH})_1\text{TPPH}_2$ and $(p\text{-NH}_2)_1\text{TPPH}_2$ shorter than $(p\text{-COO}^-)_1\text{TPPH}_2$ and TPP. Under argonated conditions the triplet lifetimes are all about 80 μs, which agrees with previous studies of TPP and tetrasubstituted TPP in nitrogen-flushed organic solvents.[46] Although comparable results are obtained for porphyrins in organic solvents and micelle environments purged with inert gases, it should be noted that the triplet excited state may still be quenched by residual oxygen.

The observed rate constant ($k_{obs} = 1/\tau_{obs}$) for the bimolecular quenching process of triplet-state photosensitizer with ground-state oxygen can be expressed as

$$k_{obs} = k_i + k_q[O_2] \quad (1)$$

where $k_i(1/\tau_i)$ is the rate constant of the intrinsic relaxation process that corresponds to the observed rate constant determined anaerobically, and k_q is the rate constant of collisional quenching due to O_2. For long intrinsic lifetimes as was the case for the porphyrins studies here, k_i becomes negligible. The reported k_i for TPP in cyclohexane is 1.6×10^3 s^{-1}, which is much smaller than $k_q[O_2]$.[53] Therefore, it is a reasonable approximation for the bimolecular rate constant to be expressed as

$$k_{obs} \approx k_q[O_2] \quad (2)$$

The bimolecular quenching constant, k_q, was calculated using the observed aerobic rate constants determined experimentally. Oxygen concentrations used in calculating the k_q values presented in Table 1 were 2.3 mM for cyclohexane[54] and 0.77 mM for micellar solutions[55,56] under aerated conditions. The latter value reflects the higher oxygen concentration found in the interior of micelles, which is approximately 3 times higher than that found in the surrounding water, 0.265 mM.[54,55] In reality there exists a solubility gradient for oxygen with the highest solubility in the hydrophobic core and the lowest at the micelle/water interface.

Discussion

The average location of the porphyrins in the aqueous micelle solution is important for photodynamic therapy, because the location and any aggregation within the system can significantly affect the photophysics of porphyrins and related compounds[57,58] and the location of the photosensitizer within cell membranes and subcellular compartments is linked to its activity and efficacy.[2,12,22,37] Previous studies have shown porphyrins that are allowed to freely diffuse into micellar solutions can (1) remain dissolved in the bulk water phase, (2) incorporate into the palisade layer of TX-100 micelles, which is a shell about 25 Å thick composed of the polar and uncharged headgroups,[59] or the Stern layer for TTAB micelles, which is a shell of the charged headgroups and is approximately 8 Å thick, [60] (3) incorporate into the hydrophobic core composed of alkyl surfactant chains,[59] or (4) intercalate between the Stern/palisade

layer and the hydrophobic core. The porphyrins studied here are water insoluble, eliminating the first possibility. The spectral features in the UV−visible and fluorescence spectra for the four porphyrins in cyclohexane, TTAB, and TX-100 are nearly identical, which leads us to conclude that the porphyrins are incorporated as monomers into a hydrophobic environment within the micelles. Nuclear magnetic resonance studies on these systems at higher concentrations provide support for the idea that localization of the porphyrins is substituent dependent as well as dependent on the specific detergent.[26] NMR data for $(p\text{-}NH_2)_1TPPH_2$ and TPP in TTAB are not available, but where available, NMR conclusions agree with those drawn from transient absorption regarding localization sensitivity to substituent and micelle type.

Effect of TX-100 Micelles on Porphyrin Triplet-State Lifetimes. Aqueous solutions of TX-100 were found to lengthen porphyrin triplet lifetimes as compared to cyclohexane solutions under aerated conditions. In particular, observed triplet lifetimes in aerated TX-100 were 1.36 μs for TPP, 2.1 μs for $(p\text{-}NH_2)_1TPPH_2$, 2.2 μs for $(p\text{-}COO^-)_1TPPH_2$, and 2.2 μs $(p\text{-}OH)_1TPPH_2$, as compared with 210−235 ns for all porphyrins in cyclohexane. The cyclohexane lifetimes are similar to those for TPP in toluene−water emulsions.[61] Similarities in the lifetimes for substituted porphyrins in TX-100 can be attributed to their average location within the micelle entity. The micelle has an amphipathic, poly(ethylene oxide) headgroup, which can solubilize the porphyrin near the aqueous phase, which has a lower oxygen concentration than the core of the micelle. The idea that the poly(ethylene oxide) headgroups function to solubilize the porphyrin near the aqueous phase is supported by the observation of similar triplet lifetimes for pyrene in TX-100 and also for pyrene solubilized using poly(ethylene oxide).[62] This work strongly suggests that the environment surrounding the porphyrins in TX-100 is poly(ethylene oxide) like. Unfortunately, we were unable to solubilize porphyrins in poly(ethylene oxide) alone to confirm this effect directly in this case. The presence of such a detergent headgroup effect would serve to explain why little or no substituent effect was seen for the porphyrins in TX-100, because if all the porphyrins localize in a similar polar environment in TX-100, the presence or change in polarity of a substituent would be expected to have a small effect. The transient absorption data agree with NMR results for $(p\text{-}COO^-)_1TPPH_2$, $(p\text{-}OH)_1TPPH_2$, and $(p\text{-}NH_2)_1TPPH_2$, which show substituent charge and polarity do not affect localization in the TX-100 system and that on average the porphyrins are located near the water−micelle interface. Although NMR results are not available for TPP, considering the lack of a polar substituent, TPP is likely to be located further into the nonpolar, oxygen rich region of TX-100, which agrees with the shorter triplet lifetime.

Effect of TTAB Micelles on Porphyrin Triplet-State Lifetimes. The observed lifetimes in TTAB are longer than in cyclohexane but are consistently shorter than those observed for the same porphyrin in TX-100. It is unlikely that this results from differences in oxygen concentration in the interiors of these two micelles because nearly identical values of oxygen concentration have been measured in quite different detergents.[55] A more likely factor to be responsible for the longer lifetimes seen in TX-100 as compared to TTAB is the difference in headgroup for these two detergents and therefore the location of the porphyrins in the different micelles. TTAB has a charged, tertiary ammonium headgroup, which would be expected to be relatively inhospitable to the hydrophobic porphyrin macrocycle and would cause the macrocycle to localize nearer the oxygen rich core region. The micelle headgroup would also be expected to interact differently with the different substituent charges and polarities. The observed effect of the micelle and charged headgroup on the triplet lifetimes was to lengthen them as compared to cyclohexane and in a manner somewhat more sensitive to porphyrin substituent than observed in TX-100. Specifically, the triplet lifetimes in aerobic TTAB are, in order of decreasing substituent charge and polarity, $(p\text{-}COO)_1TPPH_2$ $\tau_T = 1.9$ μs, $(p\text{-}OH)_1TPPH_2$ $\tau_T = 1.7$ μs, $(p\text{-}NH_2)_1TPPH_2$ $\tau_T =$ 1.6 μs, and TPP $\tau_T = 0.86$ μs as compared to 210−235 ns in cyclohexane. The order of decreasing triplet lifetime gives an idea as to the location of the porphyrin in the TTAB micelles; for example, the longest triplet lifetimes would correspond to a location nearer the water−micelle interface in a region of lower oxygen solubility and the shortest would correspond to a location closer to the nonpolar core. Similar results and conclusions were found for diprotonated TPP (H_2TPP^{2+}) in aerated toluene−aqueous H_2SO_4 microemulsions, where the lifetime of triplet H_2TPP^{2+} was found to be longer than that of TPP.[61] The results were interpreted to mean that H_2TPP^{2+} resides on the aqueous side of the interface and that the quenching of triplet H_2TPP^{2+} originated from oxygen of lower concentration in the aqueous phase rather than from the higher concentration of oxygen in the toluene droplet, whereas the TPP resides inside the droplet and quenching of triplet TPP was from the oxygen concentrated in the toluene droplet.

Effect of Micelles on Quenching Rate Constants. Micelles have been found to lengthen the triplet lifetimes of all porphyrins studied here in comparison to cyclohexane under aerated conditions. For example, the observed triplet lifetime for TPP was 0.86 μs in TTAB and 0.21 μs in cyclohexane. Using the approximations and oxygen concentration previously discussed, the bimolecular quenching constant, k_q, for TPP was calculated to be 1.5×10^9 s^{-1} in TTAB and 2.1×10^9 s^{-1} in cyclohexane, which is similar to the literature value of 2.7×10^9 s^{-1} for TPP in cyclohexane.[53] Calculated values for k_q and triplet lifetimes are summarized in Table 1. The difference in the k_q value, can be explained in part by the difference in viscosity of the solvents, because

$$k_q \sim (^1/_9)8RT/3\eta = {}^1/_9 k_{\text{diffusion}} \qquad (3)$$

where the factor $^1/_9$ is a spin statistical factor, R is the ideal gas constant, T is the absolute temperature, and η is the viscosity of the solvent.[61] Although the viscosity of the hydrocarbon core of TTAB and TX-100 is not reported, the hydrocarbon tails that comprise the core are believed to be fluidlike and comparable to viscosities of similar length alkyl chains.[39] NMR studies clearly reveal that the porphyrins in micelles are less mobile than in organic solvents, as evidenced in T_1 and T_2 relaxation times.[26] The quenching constants calculated from eq 3, using a viscosity of $\eta = 0.625$ mPa s[63] for cyclohexane and $\eta \sim 3.032$ mPa s[63] for micelles (the viscosity of hexadecane, a straight chain 16 carbon alkane), are 1.2×10^9 M^{-1} s^{-1} in cyclohexane and 2.2×10^8 M^{-1} s^{-1} in hexadecane. These values range from one-fifth to one-half lower than experimental values, which leads us to believe other factors act to reduce k_q in micelles. One possible factor is that the porphyrins are sampling variable oxygen concentrations in the micelles based on their location, rather than the constant oxygen concentration used in calculations of experimental k_q.

Conclusion

We have studied the effect of micelles on the excited triplet-state lifetimes and the bimolecular quenching dynamics with

oxygen of water insoluble TPP, $(p\text{-}COO^{-})_1TPPH_2$, $(p\text{-}OH)_1$-$TPPH_2$, and $(p\text{-}NH_2)_1TPPH_2$ in different solutions and in cyclohexane. The UV−visible and fluorescence spectra observed were indicative of monodispersed porphyrin being incorporated into the micelles. Porphyrin localization within micelles was observed to be sensitive to both substituent and micelle type. We observed longer triplet lifetimes in micelles than in cyclohexane which were indicative of varying oxygen solubilities in the different systems. The local viscosity of micelles and membranes, as well as the photosensitizer substituent, need to be considered as factors that can affect photosensitizer triplet lifetimes and bimolecular reaction rates with oxygen and therefore the efficiency of singlet oxygen production and their efficacy as PDT agents.

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