Exploratory Study of the Application of Transmission and Diffuse-Reflectance Laser Techniques in the Study of Free Radical Processes in Vesicles[1]

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Diphenylmethyl radicals generated photochemically from 1,1,3,3-tetraphenylacetone (TPA) or 1,1-diphenylacetone (DPA) have been examined in vesicles using laser flash photolysis techniques. Experiments in small vesicles generated by sonication show large magnetic field effects on the ground-state radical decay when TPA is the precursor, but not in the case of DPA. The results are interpreted in terms of rapid separation of the geminate triplet radical pair when DPA is the radical precursor. No magnetic field effect was observed on the decay of the excited radical pair formed from TPA, an observation that contrasts with results in micelles. In large (injected) vesicles, experiments with DPA and TPA demonstrate that time-resolved diffuse reflectance can be a very powerful technique in the study of opaque solutions and this technique could possibly be applied to cell suspensions. Contrary to small vesicles, no magnetic field effect was observed for the ground-state radical decay when TPA was the precursor. These differences are suggested to be related to the higher degree of rigidity of the large vesicles. Oxygen scavenging experiments suggest that access to radicals in the bilayer is more facile in the case of the small vesicles, as compared with large vesicles prepared by injection, where radical trapping by oxygen is slower than in homogeneous solution.

Introduction

Radical pair dynamics are reasonably well understood in micellar solution but our understanding of their behavior in other organized media is extremely limited. In the case of diphenylmethyl radicals, which are the subject of this work, time-resolved studies have concentrated on micelles,[6-8] solids supports,[9,10] and, to a lesser degree, on other liquid media[11] and some films.[12]

The numerous studies of radicals in micelles probably reflect the simplicity of these systems as well as the fact that their solutions are sufficiently transparent to be studied by transmission techniques such as laser flash photolysis. The behavior of triplet radical pairs in micelles is very sensitive to the application of moderate magnetic field; thus, magnetic field effects provide a way of controlling free radical behavior and a tool that can be employed to probe the properties of the local environment sensed by the radicals.[13]

Vesicles are formed from phospholipids or synthetic amphiphiles that form a bilayer in which the charged head groups are exposed to the internal entrapped water core and the outer water phase. Thus, vesicular systems have three well-defined regions: the entrapped water core, the bilayer, and the water phase exterior to the vesicles. Vesicles are frequently employed as membrane mimetic systems and they can be described as bidimensional fluids, as lateral diffusion of phospholipids or amphiphiles is much faster than flip-flop between the outer and inner layers of the bilayer. Vesicles also exhibit a phase transition; the fluidity of the bilayer increases dramatically above the phase transition temperature.[14,15] Compared to micelles, which are flexible and dynamic aggregates, vesicles have a more defined and organized structure. This higher organization of vesicles prompted us to investigate the dynamics of radicals in this macromolecular system.

Synthetic surfactant vesicles are characterized by having excellent kinetic stability.[14] While solutions of small vesicles produced by sonication are sufficiently transparent to allow the study of transient phenomena by transmission techniques, the larger and more stable "injected" vesicles have a "milky" appearance and scatter light extensively. In the case of some liquid crystals (which are also rather opaque) Leigh et al.[16] have shown that by reducing the optical path it is sometimes possible to use conventional laser photolysis in the study of these systems. In the case of large (e.g. 5000 Å in diameter) vesicles we found that this approach was inadequate. We note that since scattering increases at shorter wavelengths, the problem is aggravated at the wavelengths required in this work. However, during the last decade Wilkinson's group have pioneered the use of time-resolved diffuse reflectance in the study of transient phenomena in opaque systems.[17,18] Of direct relevance to the subject of this paper are our recent studies of the dynamics of diphenylmethyl radicals

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on zeolites and silica gel.[9,10] We find that the technique of laser flash photolysis–diffuse reflectance is readily applicable to the study of dynamic processes in large vesicles.

In this paper we report the results of an exploratory study of the photodecomposition of 1,1,3,3-tetraphenylacetone (TPA) and 1,1-diphenylacetone (DPA) in dioctadecyldimethylammonium chloride (DODAC) vesicles. The vesicles were prepared by sonication and by injection, techniques that yield unilamellar vesicles with diameters around 300 and 5000 Å, respectively.[19] The dynamics of ground-state and excited-state diphenylmethyl radicals in the absence and presence of magnetic fields was examined. The excited state of diphenylmethyl radicals has a lifetime of ca. 250 ns in many organic solvents[20] and the excited radical provides an additional handle in the study of these systems. The dynamic behavior of triplet radical pairs in vesicles is affected by the high degree of organization of these systems.

Experimental Section

Chemicals. TPA was prepared as described earlier.[20] DPA (Aldrich) was recrystallized from ethanol and dioctadecyldimethylammonium bromide (DODAB) (Eastman Kodak) was recrystallized 6 times from acetone. DODAC (dioctadecyldimethylammonium chloride from Herga Industrias Quimica, Brazil) was dried for 4 h under vacuum, extracted with ethyl ether for 72 h in a Soxhlet apparatus, and then recrystallized 6 times from acetone. Chloroform (Fisher Scientific) and sodium bromide (Anachemia) were used as received. Water was purified by passage through a Millipore Milli-Q system.

Preparation of Vesicles. Small vesicles were prepared by sonication and large unilamellar vesicles by the injection method.[19] The vesicles were prepared the same day they were used for experiments.

Small Vesicles. Between 30 and 50 mM DODAC or DODAB containing the appropriate amount of DPA or TPA (1 mM) in 5–10 mL of water was sonicated for five 3-min periods intervened by 1 min resting periods using a Branson Sonifier Model 450 sonicator with the power control positioned between 4 and 5. The temperature was maintained between 40 and 70 °C. The solution was centrifuged (10–15 min at 5000 rpm) in order to remove titanium residues.

Large Vesicles. A 20 mM DODAC or DODAB solution (usually 5 mL) containing 2 mM DPA in chloroform was slowly injected (0.278 mL/min) with a syring pump into 10 mL of water at 73–75 °C. After the injection the solution was kept at the injection temperature for another 35 min to ensure complete chloroform evaporation. Nitrogen was continuously bubbled through the solution.

Equipment. The samples (1 or 2 mL) were contained in Suprasil cells constructed of 3×7 mm^2 (transmission) or 7×7 mm^2 (diffuse-reflectance) tubing and deaerated by bubbling nitrogen for 15 min. The transmission laser flash photolysis equipment was described earlier.[20,21] To allow room for the magnet the samples were irradiated with Lumonics TE-860-2 or EX-510 excimer lasers (308 nm, ~5 ns, ≤40 mJ/pulse) from the front face at a ca. 20° angle to the monitoring beam. The diffuse-reflectance laser flash photolysis system at NRC is largely based on similar instrumentation develop by Wilkinson and co-workers[18] and has been described elsewhere.[22] The fourth harmonic of a pulsed frequency quadrupled Lumonics Nd/YAG laser (266 nm, ~12 ns, ≤40 mJ/pulse) was employed for sample excitation. The magnetic fields were supplied by the same home-built magnet described earlier.[23] All experiments were performed at room temperature (~20 °C), which is below the phase transition temperature of DODAC. The last group of experiments was performed at the University of Ottawa employing the fourth harmonic from a Continuum Surelite Nd/YAG laser for excitation. The rest of the system is similar to that at NRC, except for the use of Macintosh-based Labview software to control the experiments. The details of this laser system will be reported elsewhere.

Results and Discussion

The free radical literature provides a virtually unlimited choice of free radical sources. Our choices of TPA and DPA reflect our past experience with these precursors and the ease of characterization of the diphenylmethyl radical.[20] In both cases the lifetimes of the excited singlet and triplet states are extremely short. Photolysis of DPA leads to the formation of a diphenylmethyl and an acyl radical. In the case of TPA, $Ph_2CHC(O)^\bullet$ decarbonylates rapidly and leads to the formation of two diphenylmethyl radicals. Reactions 1 and 2 illustrate these processes.

$$\underset{\text{(TPA)}}{Ph_2CHCOCHPh_2} \xrightarrow{h\nu} Ph_2CHC\dot{O} + Ph_2CH^\bullet \qquad (1)$$

$$Ph_2CHC\dot{O} \rightarrow Ph_2CH^\bullet + CO$$

$$\underset{\text{(DPA)}}{Ph_2CHCOCH_3} \xrightarrow{h\nu} CH_3C\dot{O} + Ph_2CH^\bullet \qquad (2)$$

In both cases radical generation takes less than 10 ns and yields a triplet radical pair. In reaction 2 the acetyl radical is not expected to decarbonylate in the time scale of our experiments. In the case of micelles it has been shown that reaction 1 yields a pair of highly hydrophobic and not very mobile radicals which favor geminate processes.[6-8] In contrast, in the case of reaction 2 the acetyl radical is highly mobile and in micelles the radical pairs separate quantitatively; as a result geminate processes are not of importance for DPA.[7] Thus, by comparing TPA and DPA it is frequently possible to evaluate the importance of geminate reactions.

Magnetic fields affect the behavior of triplet geminate radical pairs. Application of a moderate magnetic field leads to the splitting of the triplet sublevels (Zeeman effect) such that the T_+ and T_- sublevels are no longer near-degenerate with the singlet state of the radical pair. As a consequence triplet–singlet interconversion of the T_+ and T_- sublevels is slowed down or shut-off. In a micellar system magnetic fields are not expected to affect the rate of radical exit from the micelle. Thus, the partition between spin evolution and micellar exit changes in favor of the latter as a result of the slow down mentioned above, and for most radical pairs escape processes play a significant role in a magnetic field. Consequently, magnetic field effects provide a suitable tool to investigate geminate behavior and to differentiate geminate from random radical encounters.

Diphenylmethyl radicals are produced in high yields, have an extinction coefficient around 80 000 M^{-1} cm^{-1} at 330 nm[24] and are easily detectable employing transient techniques.[20,25] Excited-state diphenylmethyl radicals can be detected by absorption or emission spectroscopy. In particular, their intense fluorescence is readily detectable around 530 nm.[20] We note that while excitation of the radical is a two-photon process, the extinction coefficient of the radical is sufficiently high at 308 and 266 nm (the

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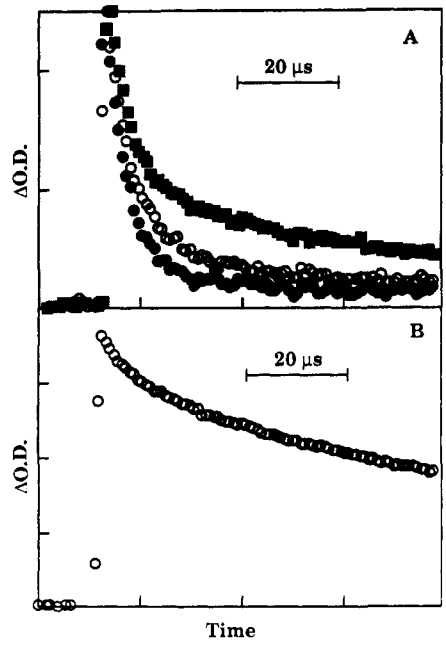

Figure 1. (A) Effect of a magnetic field on the diphenylmethyl radical decay (monitored at 330 nm) in the TPA/DODAC system (small vesicles): (●) $H = 0$; (○) $H = 1200$; (■) $H = 5000$ G. (B) Diphenylmethyl radical decay (monitored at 330 nm) in the DPA/DODAC system (small vesicles).

laser wavelengths) that a significant fraction of the radicals produced in reactions 1 or 2 are excited within the duration of the laser pulse, particularly at 308 nm.

Dynamics of Ground-State Diphenylmethyl Radicals. Small Vesicles. The experiments described in this section could be reproduced qualitatively for different vesicle preparations, but the observed decay lifetimes varied by as much as a factor of 2 for different preparations. This is probably due to the formation of vesicles with different sizes. The experiments described below are related to one particular vesicle preparation. Photolysis of TPA in DODAC vesicles leads to readily detectable diphenylmethyl radicals that decay with predominant first-order kinetics and a lifetime of ca. 5 μs, as shown in Figure 1A. This lifetime is longer when compared to the decay of the same radical pair in micelles ($\tau = 0.21$ μs in sodium dodecyl sulfate or 0.84 μs in cetyltrimethylammonium chloride).[7]

In all our experiments radical decay revealed a small deviation from first-order kinetics. This is not surprising considering that vesicles are microheterogeneous assemblies where multiple incorporation sites can be anticipated. The decay traces do not return to the pre-excitation level; for example in the trace of Figure 1A a small residue of ~5% is observed. These residual absorptions are usually attributed to radical escape and loss of geminate character. That is, once separation has occurred further radical–radical reactions rely on diffusional random encounters, these being rather slow in comparison with geminate decay. In micellar systems we have shown that in the case of diphenylmethyl radicals these residuals are further complicated by two-photon processes which tend to induce residual absorptions which do not necessarily reflect radical escape.[7]

Escape and two-photon processes of the type mentioned above can be readily differentiated by studying the power dependence of the residual absorption. Two-photon processes become negligible at low laser doses or when the data are extrapolated to "zero" dose. Indeed, in the case of micelles no residual absorption is detected at low laser doses.[7] The same behavior was observed in the case of TPA in DODAC vesicles. Thus, the decay processes observed are due to geminate reactions and radical escape is insignificant in the absence of a magnetic field.

Magnetic field studies were carried out as outlined briefly in the Experimental Section and as reported in earlier contributions from this laboratory.[23,26] Application of magnetic fields led to an increase in the radical lifetimes as measured from the traces recorded at 330 nm. Figure 1A illustrates the effect of 1200 and 5000 G fields on the decay kinetics. Saturation of the magnetic field effect is observed at ~4000 G. As mentioned in the introduction the increase of the lifetime is attributed to a decrease of the intersystem crossing in the geminate process leading to radical escape. In related systems, such as micelles of various sizes,[26,27] as well as microemulsions,[11] it has been generally observed that the magnetic fields required to influence radical-pair decay are smaller for the larger aggregates, reflecting a smaller spin exchange interaction as the separation distance increases. The relatively high fields required in vesicles suggest a greater interaction between the spins. We believe that this should be attributed to the highly structured environment in the vesicle bilayer which probably limits the separations that the radicals can achieve. Thus, in spite of the large size of vesicular aggregates, the volume explored by the radical pair is probably smaller than in the case of micelles. This, in turn, is indicative of a highly structured environment.

Even at high fields (see Figure 1A) the traces reveal a fast decay component which accounts for a significant fraction of the decay. This behavior is probably due to the contribution from T_0 which is expected to be field independent[28] and therefore about one-third of the radical pairs (assuming equal initial populations) will still decay with a lifetime around 5 μs. This behavior is only inhibited in systems involving rapid interconversion between the triplet sublevels, which is not expected for carbon-centered radicals in the absence of heavy atoms.[23]

No magnetic field effects were observed for DPA which typically showed complex kinetic behavior and half-lives in excess of 40 μs (Figure 1B). The absence of magnetic field effects and slow decay indicate that separation of the radical pair is much faster for DPA than for TPA and is consistent with the high mobility expected for the acetyl radical. The dominant decay mode in the DPA/DODAC system should be attributed to random encounters between radicals. However, the experimental traces reveal a small contribution from a rapid initial decay; this may be attributed to a minor contribution from geminate processes indicating that acetyl radical exit from the vesicles is somewhat slower than in the case of micelles,[7] thus allowing geminate decay to play a minor but detectable role. This contribution is too small to expect any magnetic field effect on the overall decay and further analysis of this small component would be very speculative. We note that the lifetimes of the slower component for the DPA/DODAC system are comparable to the slow component for TPA in the presence of a magnetic field (compare parts A and B of Figure 1). This is consistent with the fact that radical recombination involving random encounters is always the dominant process for DPA, while in the case of TPA its contribution is dominant only in the presence of a magnetic

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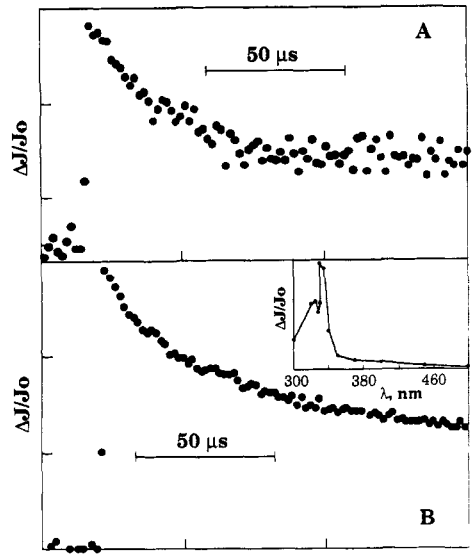

Figure 2. (A) Diphenylmethyl radical decay (monitored at 330 nm) in the TPA/DODAC (big vesicles) system. (B) Diphenylmethyl radical decay (monitored at 330 nm) in the DPA/DODAC (big vesicles) system. Inset: Transient spectrum of diphenylmethyl radicals.

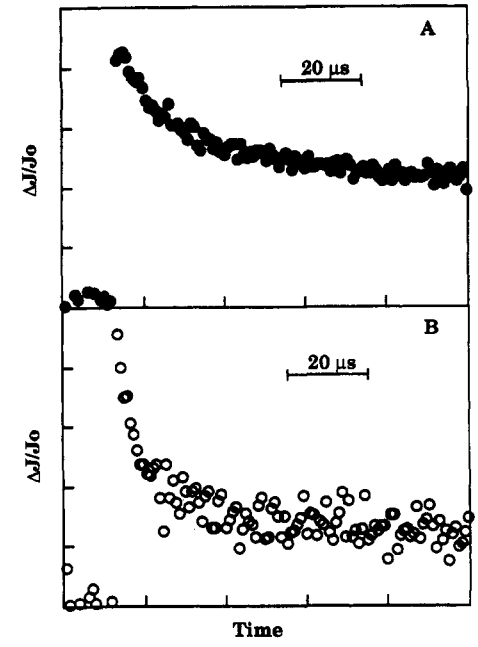

Figure 3. Decay of diphenylmethyl radicals (monitored by diffuse reflectance at 330 nm) following 266-nm excitation of TPA in injected vesicles at room temperature (**A**) and at 50 °C (**B**).

field.[29] Thus, allowing for any differences that may involve acetyl radicals, the TPA and DPA are rather similar once geminate processes become relatively unimportant.

Changing the temperature from below (20 °C) to well above (~50 °C) the transition temperature for these vesicles has very little effect on the radical decay kinetics from TPA. The small acceleration of the decay observed contrasts with the extensive effects in large vesicles (vide infra) and probably suggests that small vesicles are more fluid than the injected ones even below the transition temperature.

Large Vesicles. These are very large aggregates with diameters around 5000 Å that have been characterized by electron microscopy. These vesicles are more stable and more rigid than the smaller ones discussed earlier.[19] Their light scattering properties are such that transmission laser photolysis techniques are not suitable for their study. This led us to explore and now report on the first application of time-resolved diffuse-reflectance techniques for the study of large vesicular systems. Our studies related to the magnetic field effects in these systems unfortunately have some limitations. This simply reflects an experimental restriction; at present the system geometry required for diffuse-reflectance work allows us only the use of a small magnet with which the maximum field achievable is ~2000 G.

Laser excitation (266 nm) of DPA in large vesicles led to detectable signals which from their spectrum (Figure 2B, inset) can be readily characterized as due to the diphenylmethyl radical (λ_{max} 330 nm). The spectral characteristics are very similar to those observed in other heterogeneous systems, such as on silica gel and on zeolites.[9] The decay is complex (Figure 2) and no attempt was made to analyze it in detail, although the first half-life in large vesicles was around 80 μs and is comparable to the value obtained in small sonicated vesicles (~70 μs). As in the case of small vesicles, and consistent with a fast separation of the radical pairs, no magnetic field effect could be observed for DPA in large vesicles.

Laser excitation (266 nm) of TPA in large vesicles led to the detection of diphenylmethyl radicals (Figure 2A). A search at shorter times (30 ns/point, shortest time base available with diffuse-reflectance detection) did not reveal any fast transient. The transient decay is similar to that observed for DPA, although the first half-life is somewhat shorter (~50 μs). No change in the decay kinetics (slow down of the initial decay or increase of the residual absorption) was observed when a 2000-G field was applied. This suggests that the transient phenomena observed are related to random encounters of the diphenylmethyl radicals. It should however be noted that these vesicles are sufficiently large that multiple radical pairs may be generated in each vesicle during laser excitation. Thus, in this case "random encounters" may simply involve radicals generated by independent photochemical events within the same vesicle.

Interestingly, increasing the temperature from room (~20 °C) to 50 °C led to a marked acceleration of the decay (Figure 3). The shorter decay now reveals a significant fraction of residual absorption which decays in the long time scales observed at room temperature with TPA or when DPA was employed as a precursor. We suggest that in the highly structured environment of the injected vesicles at low temperature the radicals cannot separate enough for their exchange interaction to be sufficiently small to allow facile intersystem crossing. These exchange interactions must also be large compared with the modest magnetic fields applied. At temperatures above the phase transition (this occurs in the 35–39 °C range)[19] the radicals can achieve enough separation for intersystem crossing to be more facile; interestingly, the result of this is that the viscosity changes that would normally be expected to facilitate radical separation in this case assist geminate processes as a result of their effect on spin evolution.

(29) We tried to trap $PhCH_2^{\cdot}$ radical after escape to the aqueous phase. Cupric ions are efficient scavengers of benzyl radicals in a reversible process that leads to a readily detectable $PhCH_2$-Cu^{2+} complex.[35] Unfortunately, a similar experiment using 0.05 M aqueous Cu^{2+} did not lead to a similar complex from diphenylmethyl (using DPA as a precursor), presumably because of a lower equilibrium constant in this system. As a result it was not possible to employ this technique as a test for radical exit into the water.

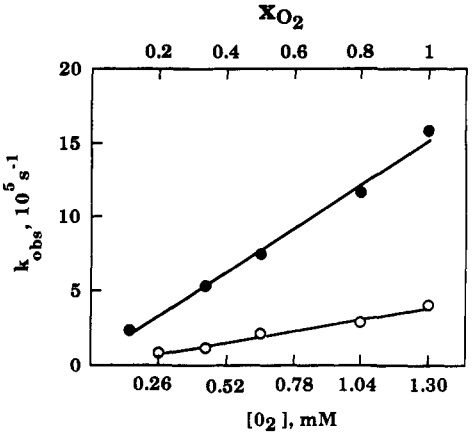

Figure 4. Plot of the observed rate constants (k_{obs}) for diphenylmethyl radical decay at ~20 °C as a function of oxygen concentration or molar fraction in the DPA/DODAC system: (●) small vesicle; (○) large vesicles.

A Comparative Study of Oxygen Scavenging in Large and Small Vesicles. To the extent that unilamelar vesicles are regarded as membrane mimetic systems,[14] the access of oxygen in these systems is of great importance. The importance of free radicals in biological and health-related problems is widely recognized, and most of these effects tend to reflect the interactions of oxygen with free radicals. We have carried out a few preliminary measurements of the reactivity of diphenylmethyl radicals toward oxygen at ~20 °C.

In order to minimize the complexities related to the geminate processes discussed before, all these measurements were carried out using DPA as a radical precursor in DODAC vesicles. Oxygen quenching plots were linear and led to the graphs of Figure 4; note that two different oxygen scales are displayed. The top one in terms of molar fraction in the gaseous O_2/N_2 mixture at 1 atm is related directly to the experiment, while the concentrations in the lower scale assume that the solubility of oxygen in pure water (1.3 mM[30]) can be used in vesicular systems. It is likely that oxygen tends to concentrate in the organic pseudo-phase, given the higher solubility of oxygen in organic media. From the plots in Figure 4 the apparent oxygen scavenging rates constants (k_{oxygen}) obtained are 1.2×10^9 and 3.2×10^8 M^{-1} s^{-1} in small and large vesicles, respectively. For comparison purposes the oxygen quenching rate constant was measured in a 1:1 water/ethanol solution (an oxygen solubility of 5.7 mM, the average between the solubility in pure water and ethanol[30] was assumed). The value obtained (7.7×10^8 M^{-1} s^{-1}) is similar to the one previously measured in cyclohexane.[20]

The quenching rate constant in small vesicles is higher than the one observed in homogeneous solution indicating a higher solubility of oxygen in the hydrophobic phase. Although the oxygen solubility is higher in the hydrophobic phase, it is interesting that the value for large injected vesicles is smaller than the ones observed in the smaller sonicated vesicles and in homogeneous solution. The observation suggests that the microenvironment in which the diphenylmethyl radicals are formed in the large vesicles is different from the one sensed in the smaller ones. It is interesting to note that these results are in line with the data for pyrene luminescence quenching by oxygen in DODAC vesicles reported by Abuin and Lissi.[31] At temperatures below the phase transition a slower rate is observed for the quenching in large vesicles when compare with the small ones; the differences being related to the relevance of the surfactant packing. We note that DODAC vesicles undergo phase transitions in the 34–39 °C range.[19,32] All our oxygen quenching experiments were carried out at room temperature (ca. 20 °C) in the rigid environment that characterizes the bilayer properties below the temperatures indicated above.

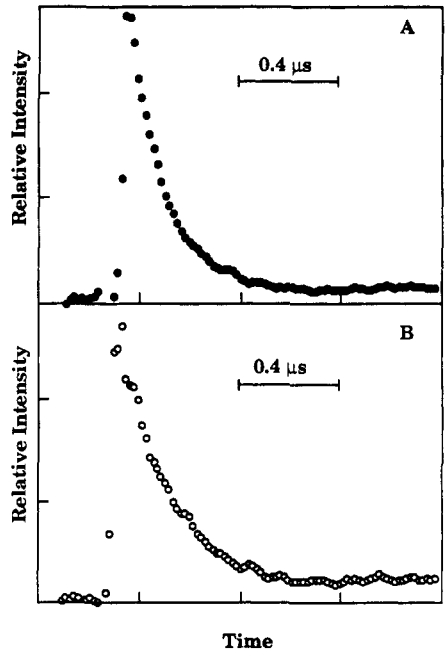

Figure 5. Fluorescence decay traces for diphenylmethyl radicals in small vesicles at room temperature, monitored at 530 nm following excitation at 308 nm: (A) TPA/DODAC and (B) DPA/DODAC systems.

Excited Diphenylmethyl Radical Behavior. As mentioned above, excited radicals are readily formed in two-photon processes during the photolysis of TPA and DPA. The formation of the fluorescent excited state of the radical reflects the rapid decomposition of the excited states of DPA and TPA (see reactions 1 and 2) as well as the absorption properties of the radical at the laser wavelength.

Small Vesicles. Excited radicals were readily detected from their fluorescence monitored at 530 nm. Figure 5 shows fluorescence decay traces in DODAC using TPA and DPA as precursors. The lifetimes are 140 and 190 ns, respectively (see Table I). Both lifetimes are shorter than that observed for the excited radical in homogeneous solution (~250 ns).[20] The lifetime of the excited radical from DPA in DODAC is even shorter than that observed in micelles (see Table I).[7] This may reflect the presence of traces of free amine impurities that may be present in DODAC. The shorter excited radical lifetime for TPA/DODAC when compared to DPA/DODAC is attributed to geminate processes in the excited radical pair, as observed previously in micelles.[7]

The lifetimes of the excited radicals formed from TPA and DPA in DODAB vesicles are 107 and 185 ns, respectively (Table I). The shortening of the lifetimes does not appear to reflect bromide ion quenching of the excited state, since addition of 20 mM bromide to DPA or TPA in DODAC did not induce any shortening of the excited radical lifetime. In contrast, in the case of micelles, shorter excited-state lifetimes in the presence of

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Table I. Fluorescence Lifetimes for Excited Diphenylmethyl Radicals Generated under Different Experimental Conditions

system	aggregate type	τ, ns
TPA/DODAC	sonicated vesicle	140
DPA/DODAC	sonicated vesicle	190
TPA/DODAB	sonicated vesicle	107
DPA/DODAB	sonicated vesicle	185
TPA/DODAC	injected vesicle	190
DPA/DODAC	injected vesicle	260
TPA[a]	cyclohexane (homogeneous)	255
TPA/CTAC[b,c]	micelles	140
DPA/CTAC[b,c]	micelles	254
TPA/CTAB[b,d]	micelles	127
DPA/CTAB[b,d]	micelles	194

[a] From ref 20. [b] From ref 7. [c] CTAC = cetyltrimethylammonium chloride. [d] CTAB = cetyltrimethylammonium bromide.

bromide counterions have been attributed to bromide quenching of the excited radical.[7] Assuming similar solubilization environments for DPA and TPA, one must assume that excited radical quenching by bromide cannot be the cause of the short lifetime (107 ns) in the TPA/DODAB system. An alternative and perhaps more likely explanation for the shorter lifetime in DODAB is that the heavy atom counterion relaxes spin selection rules and enables chemical reactions within the geminate radical pair (i.e. by promoting intersystem crossing). Note that this suggestion involves a spin interaction with the *radical pair*, rather than just with the excited radical.

Application of a 4000-G field had no effect on the decay of excited radicals from either the DPA/DODAC or the TPA/DODAC system. In micelles, where hyperfine coupling is the dominant intersystem crossing mechanism, the application of a magnetic field leads to an increase of the excited radical lifetime due to a slow-down of the triplet–singlet interconversion in the excited radical pair due to Zeeman splitting of the triplet sublevels.[7] The shorter excited radical lifetime for the TPA/DODAC system when compared to DPA/DODAC suggests the occurrence of geminate processes in the former (vide supra), but the lack of magnetic field effects suggests that hyperfine coupling is not the mechanism for intersystem crossing. This difference between vesicles and micelles is probably related to the higher viscosity and higher degree of organization in vesicles and is consistent with only very limited separation during the lifetime of the excited radical. Due to the higher viscosity and slower separation of the radical pair, a mechanism that operates at short distances and is magnetic field independent, such as spin–orbit coupling, could contribute to the intersystem crossing. This contribution would be more prominent under conditions (e.g. high viscosity, short time scales) where adequate separation for hyperfine coupling to become an effective intersystem crossing mechanism cannot be achieved. We note that a similar change in intersystem crossing mechanism has been previously observed for biradicals.[28] Because of its longer lifetime the ground-state radical pair can separate far enough for hyperfine coupling to be effective and a magnetic field effect is observed on the geminate decay.

Large Vesicles. The excited radical lifetimes of DPA or TPA in large vesicles of DODAC are longer than those observed in the smaller vesicles (Table I). This increase may reflect a reduced access of impurities to the excited state or conformational restrictions imposed on the radical by a higher microviscosity of the medium. At this point we cannot differentiate between these possibilities.

Application of a 2000-G field had no effect on the decay of excited radicals from either DPA or TPA in large vesicles. As in the case of small vesicles, the lack of magnetic field effects suggests that hyperfine coupling is not the mechanism for intersystem crossing.

Conclusion

The exploratory work described in this paper illustrates for the first time the applicability of time-resolved diffuse-reflectance techniques for the study of highly scattering vesicle solutions. Free radical chemistry in these systems is dominated by geminate processes. In the case of small vesicles ground-state radical decay is affected by the application of moderate magnetic fields. The rate constant for the geminate process at zero field is slower than in micelles, and a higher magnetic field is required to achieve a significant slow-down of geminate processes. This indicates that while hyperfine coupling may play a role, the dominant effect is probably the relative large exchange interaction between the two unpaired electrons reflecting the forced close proximity of the radical partner; thus, the structural organization of vesicles has a marked effect on the dynamics of spin evolution and in turn on radical decay. No magnetic field effect was observed for the triplet radical-pair decay in large vesicles suggesting that the high degree of organization hinders the separation to distances where the hyperfine coupling mechanism becomes operative. These results show the importance of the structure of the organized system on the dynamics of radical pairs.

Excited diphenylmethyl radicals resulting from two-photon processes are readily produced upon 308-nm laser irradiation of either TPA or DPA. No magnetic field effect was observed on the geminate decay of the excited radical pair formed in the TPA/DODAC system, suggesting that the high viscosity and degree of organization in vesicles have an effect on the spin relaxation mechanism. This change in mechanism is in contrast with the dynamics of the excited radical pair in micelles where magnetic field effects are readily detected.[7]

Finally, our studies employing time-resolved diffuse-reflectance laser flash photolysis were remarkably straightforward. To the best of our knowledge this is the first time that diffuse reflectance was employed with opaque solutions, although aqueous suspensions of solid TiO_2 have been recently studied using a similar approach.[33,34] Given the similarity of solutions of large vesicles and some cell suspensions, we are currently trying to extend the use of this technique for in vivo bacterial studies.

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