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1-[4-(Trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene: Synthesis, Fluorescence Properties, and Use as a Fluorescence Probe of Lipid Bilayers†

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ABSTRACT: 1-[4-(Trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene (TMA-DPH), a cationic analogue of diphenylhexatriene (DPH), has photophysical properties that are generally similar to those of DPH. In solution the fluorescence lifetime (τ) of TMA-DPH is short (<1.5 ns), but τ increases to ~7 ns when the probe is embedded in lipid bilayers at temperatures less than the thermal transition temperature (T_c) of the lipid. The cationic charge ensures that the probe is anchored at the lipid–water interface, most likely with the DPH moiety intercalated between the upper portions of the fatty acyl chains. The profiles of changes in steady-state anisotropies (r_{ss}) and limiting hindered anisotropies (r_∞) are similar for both TMA-DPH and DPH embedded in lipid bilayers, but r_∞ values for TMA-DPH even at $T \gg T_c$ are generally >0.14, e.g., at 35 °C in 1,2-dimyristoylglycero-3-phosphocholine (DMPC) (cf. 0.03 for DPH in DMPC at 35 °C). Electrostatic interactions of the cationic probe with head groups of phospholipids do not appear to significantly influence the apparent dynamics of the probe. TMA-DPH should prove useful in the study of the dynamics of phospholipid monolayers, e.g., in native or reconsituted lipoproteins.

The popularity of diphenylhexatriene (DPH)[1] as a probe for studies of the structure and dynamics of lipid bilayers derives largely from its favorable photophysical properties; these have been reviewed in detail elsewhere (Dale et al., 1977; Lakowicz et al., 1979). Available data (Andrich & Vanderkooi, 1976) show that the DPH molecule is oriented with its symmetry axis normal to the plane of the membrane, at least in the gel phase of lipid bilayers. The absorption and emission transition moments are essentially (but not completely) collinear, one with the other and with the symmetry axis. This makes DPH an excellent probe for studies of order in the lipid bilayers since even small displacements of the symmetry axis result in depolarization of fluorescence emission which is easily detected and measured.

Diphenylhexatriene is, however, sufficiently soluble in the hydrocarbon domain of the bilayer that we cannot be sure that the location of the molecule may not change substantially when the probe is free to extensively reorientate during its fluorescence lifetime or may even undergo translational motion that could affect significantly the measured anisotropy (Engel & Prendergast, 1981). Such possibilities make it more difficult, for example, to propose models of DPH motion in bilayers. Because r_∞ values for DPH embedded in lipid bilayers at temperatures greater than the thermal transition temperature are often ≪0.1, calculations of axial depolarization values ($\langle d^x \rangle$; Eisinger et al., 1981) for DPH, so essential for energy-transfer studies in bilayers, are problematic. As Heyn (1979) has pointed out, the data from a probe anchored at the lipid interface are inherently more easily interpreted; but even the probes suggested by Heyn, namely, the parinaric acids, are still somewhat able to penetrate the membrane as the protonated (carboxyl) form.

We have sought a fluorescent probe that would at once be tethered to the lipid–water interface and yet intercalated into the lipid milieu. We have synthesized such a molecule—1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene (TMA-DPH), a derivative of DPH with a cationic moiety affixed to the para position of one of the phenyl rings. Nikitina et al. (1963) and Cundall et al. (1979) have also reported on the synthesis and fluorescence properties of derivatives of DPH, but the information provided is sparse, especially with regard to the properties of TMA-DPH (Cundall et al., 1979). We have used this derivative to examine the dynamics of lipid bilayers and show that while its photophysical properties are fundamentally similar to DPH, the patterns of motion and, by inference, the region of the bilayer reported on by TMA-DPH are quite different from that of the parent molecule, diphenylhexatriene.

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[1] Abbreviations used: DPH, 1,6-diphenylhexa-1,3,5-triene; DMA-DPH, 1-[4-(dimethylamino)phenyl]-6-phenylhexa-1,3,5-triene; TMA-DPH, 1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene; DPPC, 1,2-dipalmitoylglycero-3-phosphocholine; DOPC, 1,2-dioleoylglycero-3-phosphocholine; DMPC, 1,2-dimyristoylglycero-3-phosphocholine; LOPC, lysooleoylglycero-3-phosphocholine; ^{1}H NMR, proton nuclear magnetic resonance; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene; Mops, 4-morpholinepropanesulfonic acid.

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Materials and Methods

Cinnamyltriphenylphosphonium chloride, *trans*-cinnamaldehyde, and diphenylhexatriene were obtained from Aldrich Chemical Co., Milwaukee, WI. All solvents used for fluorescence spectroscopic measurements were spectroscopic grade and were obtained from Burdick and Jackson Co., Muskegon, MI. All other solvents were analytical reagent grade. Phospholipids were obtained from Supelco, Bellefonte, PA, and were found to yield a single spot on thin-layer chromatography; they were therefore used without further purification.

Synthesis of Reagents. Cinnamyltriphenylphosphonium chloride (12.4 g, 0.020 mol) was dissolved in 150 mL of freshly prepared 0.2 M sodium methoxide in anhydrous methanol. 4-(Dimethylamino)-*trans*-cinnamaldehyde (4.0 g, 0.031 mol) was added and the solution heated for 1 h at reflux under argon. The orange crystals that formed on cooling to 5 °C were filtered, washed with 60% methanol in water, and then dried. The product was crystallized twice from toluene, giving 4.4 g of an orange crystalline solid, mp 159 °C. Anal. Calcd for $C_{20}H_{21}N$: C, 87.27; H, 7.64; N, 5.09. Found: C, 87.41; H, 7.47; N, 4.95 (Galbraith Laboratories, Knoxville, TN).

A mixture of 1.0 g of DMA-DPH, 10 mL of methyl iodide, and 100 mL of acetone was refluxed about 16 h with an efficient condenser. The product that separated was washed thoroughly with acetone and dried. Due to low solubility, the product was difficult to crystallize. The analytical sample was crystallized from a large volume of ethanol and had mp 195 °C. Anal. Calcd for $C_{21}H_{24}N$: C, 60.43; H, 5.76; N, 3.36. Found: C, 60.15; H, 5.79; N, 3.20.

^{1}H NMR spectra were measured on a Bruker 270-MHz NMR spectrometer by Dr. P. Kroon of Merck Research Institute, Rahway, NJ. Absorption spectra were measured on a Carey 219 spectrophotometer. Mass spectral analyses were performed by Dr. Ian Jardine of the Department of Pharmacology, Mayo Foundation, on a Kratos mass spectrometer. DMA-DPH and DPH were detected by electron-impact techniques while TMA-DPH was analyzed by field-desorption methods. Corrected fluorescence spectra were recorded on an SLM spectrofluorometer. Fluorescence lifetimes (τ) were measured by the phase-modulation method of Spencer & Weber (1969) on an SLM subnanosecond spectrofluorometer. In general, a frequency of 18 MHz was employed. For studies (on τ) with both DPH and TMA-DPH, a Corning 7-54 filter was placed in the excitation path to minimize stray light from the monochromators. The excitation wavelength was 360 nm, and, in general, the fluorescence emission was isolated from Rayleigh and Raman scattering by use of a Schott KV 420 filter. Fluorescence lifetimes were always measured with a polarizer in the emission path oriented to 54.7° to eliminate effects due to Brownian rotation, and invariably a solution of POPOP in ethanol was used as a reference lifetime standard (τ_{POPOP} = 1.35 ns in ethanol; F. G. Prendergast and P. J. Callahan, unpublished results) to minimize possible artifacts due to wavelength-dependent response of the photomultiplier tubes. Differential phase lifetimes were determined by the method originally described by Mantulin & Weber (1977) as used by Lakowicz & Prendergast (1978). Values for r_{ss} were determined by the method of Weber & Bablouzian (1966); r_0 values for TMA-DPH were determined by measurement of r_{ss} for the fluorophore dissolved in propylene glycol, maintained at −65 °C, and found to be 0.390. Excitation polarization spectra were also measured for the probe dissolved in propylene glycol, but to facilitate measurement of τ, we recorded the spectrum at −40 °C (at −65 °C the turret on the lifetime instrument is difficult to turn and if forced may result in rupture of the cooled seals). Values of r_{ss} across the emission band and of TMA-DPH were measured by selecting the wavelength either with a monochromator *or* by use of interference filters. Calculations of differential tangents, log R, and r_∞ were performed as described by Lakowicz et al. (1979).

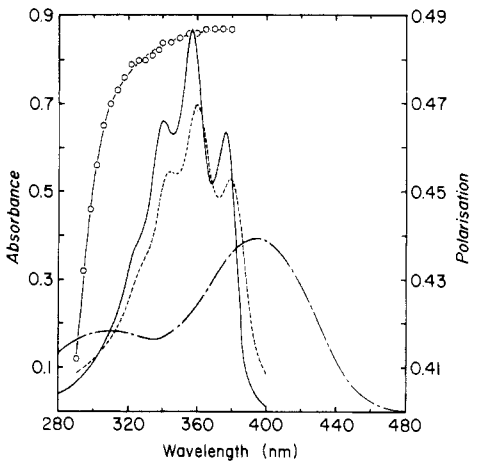

FIGURE 1: Absorption and excitation polarization spectra of DPH and derivatives. For the absorption spectra of DPH (—), TMA-DPH (- - -), and DMA-DPH (−·−), probes were dissolved to equal concentration in spectral grade dimethylformamide. The spectrum of DMA-DPH is red shifted relative to that of DPH or TMA-DPH but in acidic dimethylformamide (when the dimethylamino moiety is protonated) exhibits a spectrum that is almost identical with that of TMA-DPH, but of slightly higher extinction. The excitation polarization spectrum shown here (○) is for TMA-DPH dissolved in propylene glycol at −40 °C and is identical with that of DPH; for this spectrum only a KV 418 filter was used on the emission since there is no variation in r_{ss} with emission wavelength (see Figure 2).

Phospholipid vesicles containing either DPH or TMA-DPH were prepared as described by Lakowicz et al. (1979). Vesicles of DOPC, or those containing mixed acids (e.g., stearoyl- or palmitoyloleoylphosphatidylcholine), were annealed at 25 °C for 0.5 h; otherwise, vesicles were annealed at a minimum of 10 °C above T_c for the lipid (Lawaczeck et al., 1976). A buffer composed of 0.125 M NaCl and 0.010 M Mops, pH 7.0, was used for all experiments with vesicles or micelles.

Results and Discussion

Chemical analyses of the products have established the assumed structures of TMA-DPH, DMA-DPH, and DPH. Mass spectral data for DPH, DMA-DPH, and TMA-DPH showed the parent ion and a fragmentation pattern that was easily assigned. There was no evidence of significant impurity from materials used in the syntheses. ^{1}H NMR analysis showed resonances compatible with the chemical structure assumed for TMA-DPH. The absorption and fluorescence emission spectra of DPH and TMA-DPH are shown in Figures 1 and 2; they show that the presence of the trimethylamino group does not exert a marked effect on the basic spectral properties of the DPH moiety per se. The main effects of the trimethylammonium moiety are the marked reduction in extinction coefficient (30 200 for TMA-DPH vs. 81 000 for DPH, both in dimethylformamide) and the slight red shift of the absorption spectrum of TMA-DPH relative to DPH (Figure 1), but there are no significant differences in the emission spectra of the two chemicals. As for DPH, the absorption and emission spectra of TMA-DPH show no marked spectral shifts in response to changes in solvent polarity, but the fluorescence emission is markedly diminished by polar and/or protic media. Larger spectral perturbations would be expected from the dimethylamino derivative since the free electron pair on the

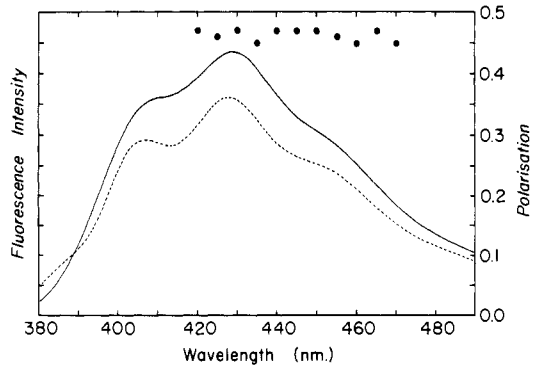

FIGURE 2: Fluorescence emission spectra of DPH (—) and TMA-DPH (---) dissolved in propylene glycol. Emission wavelength resolved anisotropies for TMA-DPH are indicated by the solid circles. Emission spectra were recorded at 0 °C while the emission wavelength resolved anisotropies were measured at −40 °C. Emission spectra for the probes in lipid bilayers were identical with those given here. The emission resolved anisotropies for TMA-DPH and DPH were identical, and the anisotropies for both were invariant with emission wavelength.

Table I: Fluorescence Lifetimes[a] for DMA-DPH and TMA-DPH in Different Solvents

	τ_ϕ in solvent					
compd	PG	DMF	CH_3CN	MeOH	EtOH	$CHCl_3$
DMA-DPH	0.51	0.50	0.34	0.41	0.57	0.80
TMA-DPH	0.37	0.16	0.21	0.27	0.21	0.14

[a] Lifetimes by phase (τ_ϕ) are given. For all lifetime measurements a modulation frequency of 30 MHz was used. A Corning 7-54 filter was used to isolate the excitation band and Schott KV 418 filters were employed on the emission to eliminate Rayleigh and Raman scattering effects. POPOP in ethanol (τ = 1.35 ns) was used as a lifetime reference. Cundall et al. (1979) did not give values for TMA-DPH, but for all other derivatives of DPH τ varied between 0.5 and 2.0 ns, approximately. Abbreviations used: PG, 1,2-propanediol; DMF, dimethylformamide; CH_3CN, acetonitrile; MeOH, methanol; EtOH, ethanol; $CHCl_3$, chloroform.

tertiary amino nitrogen is readily delocalized into the conjugated diphenylhexatriene moiety. In addition, the tertiary amine will readily hydrogen bond and, hence, not surprisingly, the spectral properties of DMA-DPH are markedly sensitive to both solvent polarity and proticity. Thus, the absorption spectrum of DMA-DPH under neutral or basic conditions is red shifted relative to that of DPH or TMA-DPH but becomes essentially identical with that of TMA-DPH upon protonation of the amino moiety; fluorescence spectra of DMA-DPH behave in analogous fashion in response to pH changes. Even more marked spectral responses have been described by Cundall et al. (1979) for other DPH derivatives; especially marked spectral shifts were shown by 1-(4-nitrophenyl)-6-phenylhexa-1,3,5-triene in response to changes in solvent polarity. In contrast, the quaternary ammonium moiety, while it must perturb the spectral properties, would be expected to do so only minimally, especially with regard to the fluorescence emission.

The emission spectra for TMA-DPH and DPH in lipid bilayers are identical one with the other and with their spectra in propylene glycol (Figure 2), and the excitation polarization spectrum of TMA-DPH is the same as for DPH, r_0 = 0.390 (Lakowicz et al., 1979). Also, as for DPH, r_{ss} is invariant across the emission band for TMA-DPH (Figure 2). In Table I, we have listed values of τ for DMA-DPH and TMA-DPH in different solvents and in DMPC at 15 °C. The most prominent feature of these data is the very short fluorescence lifetime of both derivatives, but especially of TMA-DPH in polar (and protic), polar nonprotic, and nonpolar solvents.

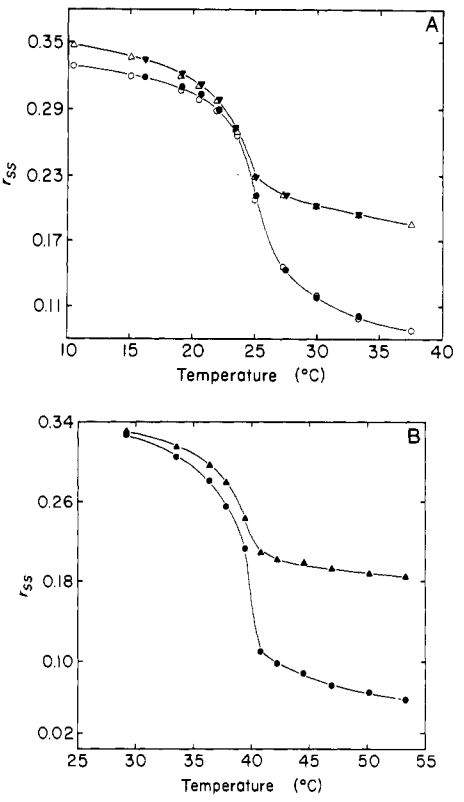

FIGURE 3: Variation in steady-state anisotropies (r_{ss}) with temperature for DPH (circles) and TMA-DPH (triangles) embedded in vesicles of DMPC (A) and DPPC (B). The solid symbols are for data taken as the temperature was increased and the open symbols as the temperature was decreased. Note, however, that all measurements were made with the temperature held constant at each point.

Indeed, the short fluorescence lifetimes led Cundall et al. (1979) to conclude that these derivatives of DPH were not likely to be of much use in biophysical studies. We did not measure quantum yields, but Cundall et al. (1979) gave values for both τ and quantum yields for derivatives of DPH. Their data showed generally low values for both parameters with all DPH derivatives. The fluorescence lifetimes reported by these authors for TMA and DMA-DPH were very similar to those given in Table I. The remarkable finding is that when TMA-DPH is embedded in lipid bilayers, the fluorescence lifetime (and presumably ϕ_f) is markedly enhanced (Table I). Cundall has verified these changes in τ when TMA-DPH is embedded in lipid bilayers (Cundall, personal communication). The explanation for these short lifetimes is not immediately apparent, especially when we would have anticipated from the behavior of DPH that τ would lengthen when TMA-DPH was dissolved in solvents of low dielectric constant, e.g., CH_3CN (Table I). The one possibility that comes to mind is that TMA-DPH *might* form micelles in aqueous solution and inverted micelles (because of the cationic charge) in media of low dielectric constant, and in either case low τ might result from interaction between the aromatic systems of the now adjacent TMA-DPH molecules (excimer formation). Iodide was the counterion in our experiments and could have been a quencher, especially in aqueous solutions, but similar lifetime behavior is found for *p*-toluenesulfonate salts of TMA-DPH. Our explanation is clearly speculative, but as yet no other reasonable explanation of the τ data for TMA-DPH in solvents has come to mind.

In most of the experiments to be presented below, measurements were made on vesicles that were prepared at the same time and in duplicate but with either DPH or TMA-DPH embedded. The data are presented in Figures 3–9 for

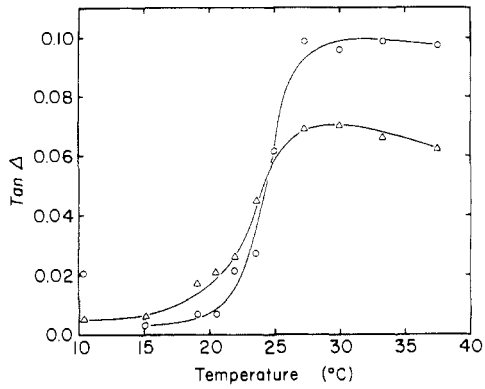

FIGURE 4: Differential tangent (tan Δ) data for TMA-DPH (Δ) and DPH (○) in DMPC vesicles as temperature is varied.

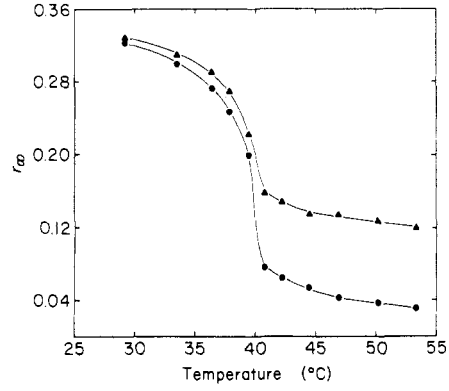

FIGURE 5: Limiting hindered anisotropies (r_∞) for TMA-DPH (▲) and DPH (●) embedded in DMPC vesicles.

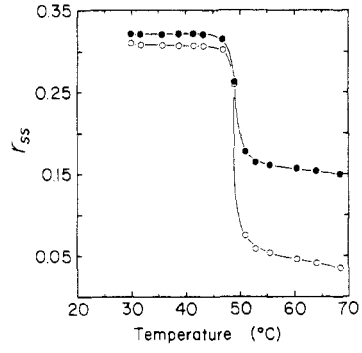

FIGURE 6: Steady-state anisotropies for TMA-DPH (●) and DPH (○) in vesicles of dimyristoylphosphatidic acid.

the two probes in a variety of lipid systems. Changes in steady-state anisotropy (r_{ss}) are shown in Figure 3 for TMA-DPH and DPH in bilayers prepared from DMPC and DPPC. The profiles are obviously similar, but r_{ss} for TMA-DPH attains a plateau very rapidly. The differential tangent data given in Figure 4 for TMA-DPH and DPH in DMPC and the behavior of the limiting hindered anisotropies for these probes in DPPC (Figure 5) show that the profiles seen in Figure 3 are not artifactual since the same pattern is observed with tan Δ and r_∞ plots. Since we may assume that TMA-DPH is tethered at the lipid–water interface and that the DPH moiety of TMA-DPH is intercalated between the fatty acyl chains [in a similar manner to the position and orientation of DPH embedded in lipid bilayers; see Andrich & Vanderkooi (1976)], then TMA-DPH should be probing essentially the glycerol backbone region and the fatty acyl chain regions probably as far down as C_8–C_{10} [notwithstanding the long-range perturbing effects the probe may exert; cf. Podo & Blaisie (1972)]. A

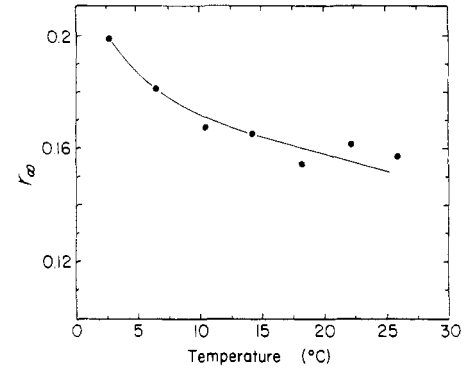

FIGURE 7: Limiting hindered anisotropies for TMA-DPH in LOPC micelles as temperature is varied.

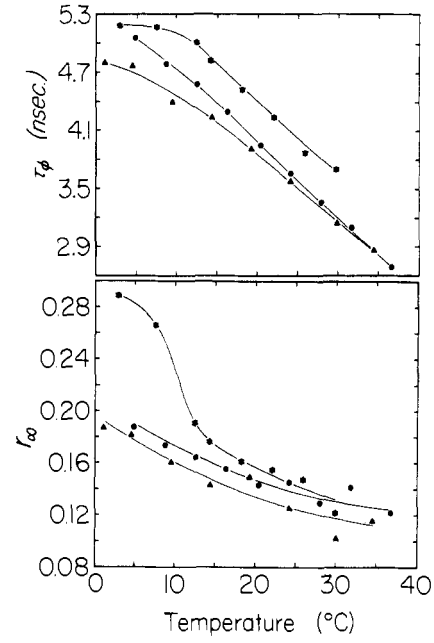

FIGURE 8: Fluorescence lifetimes (top panel) and limiting hindered anisotropies (bottom panel) of TMA-DPH in DEPC (*), DOPC (▲), and egg lecithin (●) vesicles. Data on DOPC are included here for comparison.

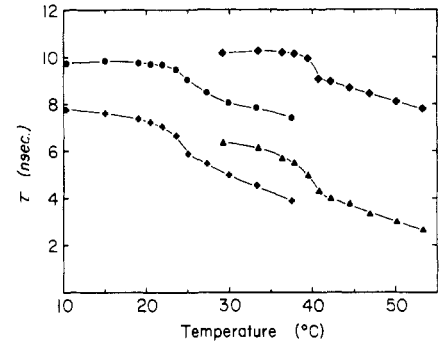

FIGURE 9: Fluorescence lifetimes of TMA-DPH in DMPC (+) and DPPC (▲) and of DPH in DMPC (●) and DPPC (♦). Only phase lifetimes are shown.

priori, there is no reason to believe that T_c should be dependent on the region of the bilayer being probed; the data presented here do not show any marked differences in T_c as detected by TMA-DPH, but clearly the limit to the motions of TMA-DPH is attained before the maximum change (decrease) in packing density of the lipid is achieved (at least as indicated by the behavior of DPH). The breadth of the transition shown by TMA-DPH therefore appears marginally narrower than that

depicted by DPH. We are unsure of the significance of this finding but suggest that the difference reflects the greater ability for DPH to redistribute in the bilayer, not only in terms of rotational motion but also through translational motion during its fluorescence lifetime (Engel & Prendergast, 1981). The difference in behavior of the two probes could, however, reflect the influence of the cationic charge of TMA-DPH on its interaction with the head groups of the phospholipid. Examination of Figure 6 reveals, however, that the r_∞ profiles for TMA-DPH and DPH in vesicles of dimyristoylphosphatic acid are the same as those observed for these probes in the zwitterionic lecithin vesicles. Our failure to detect any significant difference in the behavior of the two probes in this highly anionic lipid system argues against there being marked electrostatic influences on TMA-DPH motion.

The difference in the behavior of TMA-DPH and DPH is equally striking when the motions of the probes in unsaturated lipid dispersions are examined. These data are given in Figures 8 and 9 where we show that TMA-DPH reports a significant degree of order in DOPC and egg lecithin vesicles. However, in LOPC micelles r_∞ values for TMA-DPH are even higher than those found for the probe in DOPC; in contrast, r_∞ values for DPH embedded in these systems show the fluorescence of DPH to be essentially completely depolarized (F. G. Prendergast, unpublished data). The fluorescence lifetimes of TMA-DPH in LOPC micelles fall to very low values (ca. 0.5 ns) at high temperatures, and this may, in part, explain the apparent differences in r_∞. However, the r_∞ data given here have been calculated by using measured values for τ. Admittedly, they are not corrected for the effects of the rotation of the entire micelle on the measured fluorescence depolarization [see Engel & Prendergast (1981)]. We do not at present know the size of LOPC vesicles and hence cannot estimate their rotational correlation times in order to make appropriate corrections. We suggest that the differences observed between TMA-DPH and DPH are merely reflective of the higher degree of order of the glycerol backbone region compared to the region formed by the fatty acyl moieties of the bilayer, and this difference is most dramatic in unsaturated systems such as DOPC or LOPC. Interestingly, r_∞ values for TMA-DPH in DOPC and in saturated lipids at $T > T_c$ are asymptotic toward the same value, $\sim$0.12. The higher r_∞ values for TMA-DPH in LOPC micelles as compared to DOPC may be attributed to tighter packing in the head-group region of lysolecithin systems and provide evidence, de facto, for the proposals of Israelachvili et al. (1977) regarding the packing of lecithin phospholipids. Fluorescence lifetimes for TMA-DPH and DPH in vesicles are presented in Figures 8 and 9 and show that τ begins to decrease at T (slightly) $< T_c$ and decreases progressively through T_c and beyond. In DOPC and egg lecithin systems τ decreases in an almost linear fashion with temperature.

The changes in τ of TMA-DPH are worth further consideration. As we pointed out earlier, τ of TMA-DPH in polar or nonpolar solvents is short. The lipid bilayer obviously promotes segregation and ordering of the probe molecules and provides an apolar environment, all of which must contribute significantly to the enhancement of τ. It is not surprising, therefore, that the highest values for τ are attained for the probe in saturated lipids at $T < T_c$, but it is also apparent that in all systems at equivalent temperatures, $\tau_{\text{TMA-DPH}} < \tau_{\text{DPH}}$. We believe this to be attributable to the photophysical properties of TMA-DPH per se rather than to solvent perturbing effects but have no way of proving such a contention. However, we know that the fluorescence lifetime of TMA-DPH is very sensitive to the presence of water and other polar solvents (see above). The lifetime changes exhibited by the probe when it is embedded in lipid bilayers therefore provide us with an index of the change in accessibility of the fluorophore to the aqueous environment as temperature is varied. The validity of this assertion clearly rests on the assumption that TMA-DPH is oriented in the bilayer with its symmetry axis oriented normal to the plane of the bilayer. The assumption is obviously predicated on the behavior of the diphenylhexatriene portion of the molecule (Andrich & Vanderkooi, 1976). Fluorescence anisotropy measurements on oriented multibilayers or polarized absorption measurements are clearly needed. But we assert that the circumstantial evidence from the available fluorescence data on the changes in TMA-DPH fluorescence when the molecule is embedded in bilayers is sufficient to allow the inference we have drawn regarding the location of the probe. Thus, TMA-DPH, given its putative location, would understandably be more accessible than DPH to solvent, although the patterns of lifetime changes in the lipids (e.g., around T_c) are essentially identical. However, as for DPH, the mechanism of this increased accessibility of the probe to water cannot be assigned; i.e., it may be due either to an increase in the rate and/or extent of diffusion of water into the bilayers *or* of the probe itself into the bulk aqueous phase; alternatively both mechanisms may contribute to the observed effect. In any event, the lifetime changes by themselves cannot reasonably be used as an index of the polarity of the environment of the probe. E. Taswell, L. W. Engel, and F. G. Prendergast (unpublished results) have provided a detailed discussion of the nature and significance of changes in τ for DPH in lipid bilayers.

Finally, the fluorescence lifetimes of TMA-DPH and DPH embedded in lipid bilayers are seldom homogeneous. When phase fluorometric techniques are employed, the heterogeneity is indicated by disparity in the τ as measured by phase (τ_ϕ) and modulation (τ_m). We have performed heterogeneity analysis on lifetimes of both probes in bilayers (by measurement of τ at different frequencies) and showed the existence of a short lifetime component that accounts for a fraction of the total fluorescence that is inconstant as temperature is varied (E. Taswell, L. W. Engel, and F. G. Prendergast, unpublished results). That the observed extent of heterogeneity does not markedly affect the calculated r_∞ values has already been shown by Lakowicz et al. (1979) and Kinosita et al. (1979), who were also faced with the same problem. For this reason, no attempt was made to correct for the small disparities in τ_ϕ and τ_m found in our studies with TMA-DPH.

Conclusions

The cationic derivative of DPH that we have described is a useful probe of lipid bilayer structure and dynamics. As we shall show in the following paper, it is easier to model the motions of such a molecule in lipid bilayers, and the data are especially interesting since they may reflect the degree of order in the glycerol backbone region and upper portion of the fatty acyl chains of phospholipid membranes. Since the probe is marginally soluble in water, it should, for example, be possible to label just the outer monolayer of a unilamellar vesicle on the premise that the cationic charge would result in only slow transmembrane movement of the molecule. TMA-DPH should be especially useful, therefore, in the study of monolayer dynamics of lipoproteins or similar analogous systems. We anticipate that TMA-DPH will also be useful in energy-transfer studies in bilayers since calculations of axial depolarization factors ($\langle d^x \rangle$) are relatively easy for this probe due to the high predictability of orientation in the bilayer and the

fact that r_∞ is seldom <0.1 [cf. Eisinger et al. (1981)]. And finally, the marked differences in fluorescence properties of the probe in solution and bilayers point to some interesting and unusual photophysical properties of this molecule that bear further investigation.

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Values for and Significance of Order Parameters and "Cone Angles" of Fluorophore Rotation in Lipid Bilayers†

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ABSTRACT: A rigid formalism has been developed for the calculation of the order parameter S for fluorescence probes embedded in environments that hinder the motions of the probes and for calculation of a "cone angle" of fluorophore rotation from the order parameters. The motions of the fluorescence probes 1,6-diphenylhexa-1,3,5-triene (DPH) and 1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene (TMA-DPH) embedded in lipid bilayers were then analyzed in terms of the order parameter and the cone angle. Order parameters for such fluorescence can only be compared to the average order parameter over a segment of a fatty acyl chain or of membrane thickness. Also, because the bilayer may be perturbed by the fluorophore at regions distant from the immediate location of the probe, these "averaged" order parameters cannot be easily compared to those calculated from nuclear magnetic resonance data but are more readily compared to order parameters of electron spin resonance probes. There is no defined mathematical relation between the order parameter and a dynamical parameter which would afford a calculation of membrane "microviscosity". A Gaussian angular freedom parameter or cone angle of fluorophore motion has been calculated from the order parameters and shows in a geometric sense the limitations imposed on the angular displacements of TMA-DPH as compared to DPH.

In the preceding paper we have described the fluorescence depolarization of two molecules, diphenylhexatriene (DPH)[1] and a charged analogue thereof, 1-[4-(trimethylamino)-phenyl]-6-phenylhexa-1,3,5-triene (TMA-DPH) (Prendergast et al., 1981), when these are embedded in lipid bilayers. From these data we may infer the dynamic behavior of the probes' environment on the premise that the fluorophore does indeed provide a reliable measure of the response of its environment to a perturbation. But while we can now reasonably quantify some of the parameters of motion of probes such as DPH and TMA-DPH, the interpretation of these parameters in terms of membrane structure and mobility is not simple. We have grown accustomed, for example, to using the term "membrane fluidity" and the associated quantity "microviscosity", but the effective definition of these terms that would allow translation of one into the other is, in fact, elusive, especially in those situations where microviscosity values are determined through use of steady-state fluorescence anisotropy (r_{ss}) values. Several recent publications have shown the inappropriateness of as-

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[1] Abbreviations used: DPH, 1,6-diphenylhexa-1,3,5-triene; TMA-DPH, 1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene; DMPC, 1,2-dimyristoylglycero-3-phosphocholine; DPPC, 1,2-dipalmitoylglycero-3-phosphocholine; DOPC, 1,2-dioleoylglycero-3-phosphocholine; LOPC, lysooleoylglycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoylglycero-3-phosphocholine; ESR, electron spin resonance; NMR, nuclear magnetic resonance; PC, phosphatidylcholine.

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