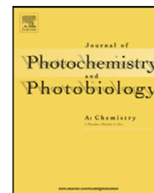
Journal of Photochemistry and Photobiology A: Chemistry 200 (2008) 381–387

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem



Unusual fluorescence spectral response of 1-(4-*N*,*N*-dimethylaminophenylethynyl)pyrene towards the thermotropic phase change in lipid bilayer membranes

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ARTICLE INFO

Article history:
Received 5 June 2008
Received in revised form 12 August 2008
Accepted 1 September 2008
Available online 10 September 2008

Keywords:
Fluorescent molecular probe
Lipid bilayer membrane
Phase transition
Partition coefficient

ABSTRACT

The applicability of 1-(4-*N*,*N*-dimethylaminophenylethynyl)pyrene (DMAPEPy), a pyrene derivative showing intramolecular charge transfer, as a prospective probe for lipid bilayer membranes has been evaluated. High sensitivity of DMAPEPy to solvent polarity and viscosity makes it to act both as a polarity-sensitive probe and as a fluorescence anisotropy probe. The molecule shows high partition efficiency towards bilayer membranes in both solid gel as well as in the liquid crystalline phases. The emission spectrum, quenching experiment and lifetime data suggest bimodal distribution of DMAPEPy in the bilayer. Using the solvent polarity scales the polarity parameters of the two locations in lipid bilayer have been estimated. In the bilayer environment it exhibits remarkable spectral changes with temperature. The thermotropic phase change of the bilayer is sensitively monitored by fluorescence intensity as well as fluorescence anisotropy parameters. DMAPEPy is also capable of sensing the changes induced by membrane modifiers like cholesterol.

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1. Introduction

Fluorescence probing has been considered as one of the most simple, convenient, less perturbing, economical techniques to study the properties of lipid bilayers [1–5]. The success of fluorescent molecular probes can be explained by the distinct advantages offered in terms of high sensitivity, selectivity, fast response time, non-invasive nature and minimal perturbation to the microenvironment to be probed. The measured changes in different fluorescence parameters of a fluorescent probe can be easily related to various molecular properties of the environment such as polarity, viscosity, diffusion coefficients, distance between groups, structural changes due to existence of different phases, formation of microstructures and microdomains, etc. Conceptually different types of fluorescent probes have been employed to study diverse properties of lipid bilayer membranes. The probes with environmentally sensitive steady-state fluorescence parameters are of particular interest and a wide variety of such fluorescent probes have been employed to meet the need. Prodan, Laurdan, Dansyl and ANS, etc. are few examples of polarity-sensitive fluorescent molecules often used as membrane probes [6–19]. However, all these probes have got their own limitations. For instance, Prodan is known to show preferential partitioning to the liquid crystalline phase of the dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) bilayers but in the solid gel phase large fraction of it remains in water [6–11]. In order to study the membrane properties it is necessary to subtract the fluorescence contribution of Prodan in water from the overall intensity, which is a serious drawback. Laurdan shows higher partitioning efficiency in both the phases but it is not possible to get information about the bilayer properties directly from its spectral behaviour and the spectral data need extensive post-experimental analysis [12–14]. Dansyl probes by themselves are not much effective and often need lipid anchors for efficient partitioning to the membrane [15–17]. ANS being a charged probe shows specific interaction in the lipid bilayer and perturbs the membrane properties [18,19]. Consequently, there is an escalating interest in designing new fluorescent molecules with superior spectroscopic properties to be used as probes for membranous studies. Phospholipid bilayers exhibit a number of phases and phase transitions with increasing temperature. Of these, the main phase transition or chain melting transition is the most important and it brings about significant changes in the membrane properties [20–23]. The physical state, organisation and function of membranes are intricately connected to the phase behaviour of lipid bilayers [24]. Owing to its physiological importance, it becomes important to device a simple, less perturbing, economical method to study the phase behaviour of lipid bilayers.

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doi:10.1016/j.jphotochem.2008.09.001

In this work, we present the novel application of a recently introduced [25] pyrene derivative, 1-(4-*N*,*N*-dimethylaminophenylethynyl)pyrene (DMAPEPy), as a proficient fluorescent probe to monitor the phase change in various phospholipid vesicles. Although the fluorescence spectra of many fluorescent membrane probes show large changes on partitioning from aqueous phase to the membrane phase, not many examples are known where there is such a large fluorescence spectral change as a response to phase change, while present in the membrane. This is first of its kind, which shows remarkable spectral response to the thermotropic changes in the lipid bilayers. It is possible to get information about the physical state of the bilayer directly from the steady-state fluorescence spectra of the molecule in the vesicles. The detailed photophysical study of this molecule has been carried out recently [26]. The design of the molecule is such that it offers the requisite structural features and functionality to the molecule to act as an environment-sensitive membrane probe. For instance (i) the presence of the conjugating ethynyl bridge between the donor (DMA) and acceptor (pyrene) facilitates the charge transfer across the molecule and results in an increased dipole moment ($\Delta\mu \approx 30$ D) in the excited state, as a result of which it exhibits a strong fluorescence solvatochromism, (ii) the hydrophobic nature and affinity towards non-polar solvents ensure its lipophilicity (the molecule is insoluble in water and needs at least 1% of EtOH (v/v) for solubilisation), (iii) the flatness of the molecule can further help in its easy incorporation into the membrane, and (iv) considerably high extinction coefficient and quantum yield in nonpolar solvents make it possible to work with very low concentration of the molecule. The sensitivity of the molecule to solvent polarity and medium viscosity [26] encouraged us to exploit it as a possible membrane probe.

2. Experimental

2.1. Materials

DMAPEPy was synthesised, details of which are given elsewhere [26]. DMPC, DPPC, dipalmitoylphosphatidylglycerol (DPPG) and cholesterol were purchased from Sigma Chemical Co. (Bangalore, India) and were used as such. Cetylpyridinium chloride (CPC) was purchased from SRL Pvt. India Ltd. and was used as received. All solvents used were of analytical grade and were all distilled before use. Triple-distilled water from alkaline permanganate solution was always used for the experiments.

2.2. Vesicle preparation

Both unilamellar (SUV) and multilamellar vesicles (MLV) were used in the studies. Unilamellar vesicles were prepared by ethanol injection method [27]. The ethanolic solution of the lipid was injected rapidly with the help of a fine needle to the aqueous medium, maintained at 50 °C (optimised condition). The volume of ethanol injected is always less than 1% (v/v) in order to avoid any damage to the vesicle by ethanol. The cholesterol incorporated lipid vesicles were prepared by solvent evaporation method. DMPC-cholesterol liposomes were prepared by adding same volume of lipid in chloroform to different volumes of cholesterol stock in chloroform; such that the molar ratio of cholesterol is varied from 0 to 50 mole% of the lipid. The solution was evaporated with the help of a rotary-evaporator and the residual solvent was removed by applying vacuum. The lipid film was left under vacuum to ensure complete dryness. Liposome vesicles were prepared by adding appropriate volumes of triply distilled water to the dry lipid film with vigorous vortexing and then warming at 10° above phase transition temperature for complete hydration. The concentration of the vesicle was varied by subsequent dilutions.

2.3. Labeling

Except for the partition coefficient experiments, the probe was always added to the lipid stock solution prior to the vesicle formation. For the partition coefficient experiments, however fixed volume of the probe was added to the vesicle of varying concentration ($0\text{–}4.5 \times 10^{-4}$ M) so that the final concentration of the probe was 1×10^{-7} M and the solution was equilibrated for an hour above the phase transition temperature. For all the experiments, a system containing the same concentration of lipid in absence of probe was used as a blank.

2.4. Fluorescence measurements

Fluorescence measurements were carried out with Hitachi F-4500 spectrofluorimeter. The excitation and emission spectra were recorded with 5/5-nm slit widths. For temperature dependence experiments the temperature was controlled by circulating water through a jacketed cuvette holder from a refrigerated bath (INSREF Ultra Cryostat, India). Temperature was also checked inside the cuvette before and after the experiments and the variation was negligible. The steady state fluorescence anisotropy (r_{ss}) values were obtained using the expression $r_{ss} = (I_{\parallel} - GI_{\perp})/(I_{\parallel} + 2GI_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$ are fluorescence intensities when the emission polarizer is parallel and perpendicular, respectively, to the direction of polarization of the excitation beam, and G is the factor that corrects for unequal transmission by the diffraction gratings of the instrument for vertically and horizontally polarized light.

2.5. Fluorescence lifetime measurements

Lifetime measurements were carried out using an IBH single-photon counting fluorimeter in a time-correlated single-photon counting arrangement consisting of ps/fs Ti-Sapphire Laser system (Tsunami Spectra Physics, Bangalore, India). The pulse repetition rate was 82 MHz and the full width half maximum is less than 2 ps. The emission was collected at magic angle polarization (54.7°) to avoid any polarization in the emission decay. The instrument response time is approximately 50 ps. The decay data were further analysed using IBH software. A value of χ^2, $0.99 \leq \chi^2 \leq 1.4$ was considered as a good fit.

3. Results and discussion

3.1. Fluorescence spectral studies of DMAPEPy in DMPC vesicles

Fig. 1 shows the emission spectra of DMAPEPy with increasing amount of lipid (0–0.45 mM). The molecule is insoluble in water and needs at least 1% (v/v) of ethanol for solubilisation. The emission of the molecule in water (1% EtOH) is very weak and with the addition of small amount of liposome (0.0125 mM) there is a remarkable increase in intensity (almost 10-fold increase) observed. The emission maximum shows a blue shift of around 16 nm on going from water (515 nm) to liposome medium (499 nm) with a small additional emission band around 427 nm in case of spectrum in liposome medium. The remarkable increase in intensity, blue shift in the emission spectrum and increase in the fluorescence anisotropy ($\lambda_{ex} = 390$ nm, $\lambda_{em} = 510$ nm) value from 0.06 in water to 0.22 in 0.0125 mM liposome confirm that DMAPEPy has been incorporated into the lipid bilayer. The emission band at 427 nm, which initially developed as a small shoulder on the 499-nm band at low concentration of lipid, becomes a structured peak

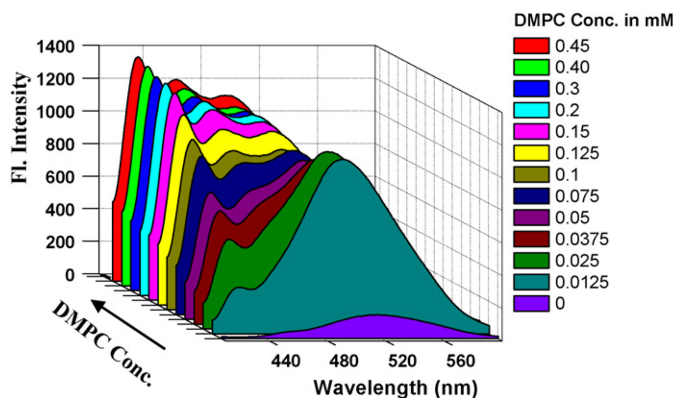

Fig. 1. Emission spectra of DMAPEPy (1×10^{-7} M) with increasing amount of DMPC unilamellar vesicles. [DMPC] = 0–0.45 mM, [DMAPEPy] = 1×10^{-7} M, λ_{ex} = 390 nm and temperature = 10 °C.

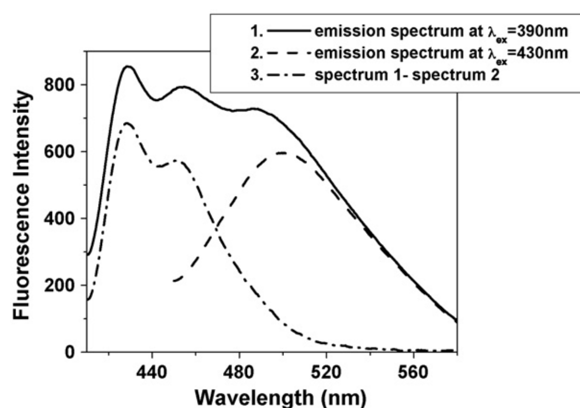

Fig. 3. Emission spectra of DMAPEPy in 0.45 mM DMPC unilamellar vesicles (temperature = 10 °C). Spectra 1 and 2 represent the emissions obtained by exciting the molecule at λ_{ex} = 390 and 430 nm, respectively. Spectrum 3 is obtained by subtracting spectrum 2 from spectrum 1.

with an additional band around 450 nm at higher concentrations of lipid. The 427 nm emission band increases in intensity and reaches saturation by 0.25 mM of liposome, whereas the intensity at 499 nm remains almost constant.

Fig. 2 represents the excitation spectra of DMAPEPy in liposome at 0.45 mM of DMPC. In liposome the excitation spectra are obtained at the two emission maxima, 430 and 500 nm. The excitation spectrum in liposome at emission wavelength 500 nm matches well with that in water (with 1% EtOH). However, the intensity in water is very weak as compared to that in liposome (inset in Fig. 2).

The excitation at emission wavelength 430 nm is similar to that in non-polar, non-interactive solvents like cyclohexane and *n*-hexane [26]. The excitation spectrum obtained at λ_{em} = 500 nm extends up to 470 nm, whereas the excitation spectrum obtained at λ_{em} = 430 nm does not have any contribution beyond 420 nm (Fig. 2). Thus, by exciting the molecule around 430 nm it is possible to get the emission profile from the fraction of probe, which shows emission maximum at 500 nm.

In Fig. 3 the broad and structureless spectrum is obtained by exciting the molecule at 430 nm. By subtracting this spectrum from the original spectrum obtained at λ_{ex} = 390 nm, it is possible to get an approximation of the emission from the fraction of probe showing the structured emission. Thus, at the highest concentration of DMPC studied (0.45 mM) the emission spectrum is a combination of two bands one structured band similar to that in non-polar, non-interactive solvent and the other broad and structureless band similar to that in polar solvents [26]. This is supported by the difference observed in the excitation spectra (Fig. 2) of DMAPEPy obtained at the two emission maxima in liposome. These experimental findings suggest the distribution of the probe between two different locations in the bilayer, one polar and the other non-polar site. Based on the emission spectra of DMAPEPy in organic solvents, the positions of the emission bands and Stokes' shift can be correlated to the dielectric constant ε (within the continuous dielectric model) or as a function of the empirical solvent polarity index, $E_T(30)$ scale [26]. Using these scales the polarity of the two locations of DMAPEPy in lipid bilayer has been estimated to be around $\varepsilon \approx 20.2$, $E_T(30) \approx 48.1$ for the polar site, which matches with the solvent isopropanol and around $\varepsilon \approx 1.9$, $E_T(30) \approx 30.9$ for the non-polar site, which matches with hydrocarbons. The fraction of DMAPEPy residing in the polar surface site can undergo intramolecular charge transfer hence showing a broad structureless emission, whereas the fraction of it in the inner non-polar site shows emission from the local excited state with an emission with vibrational structures.

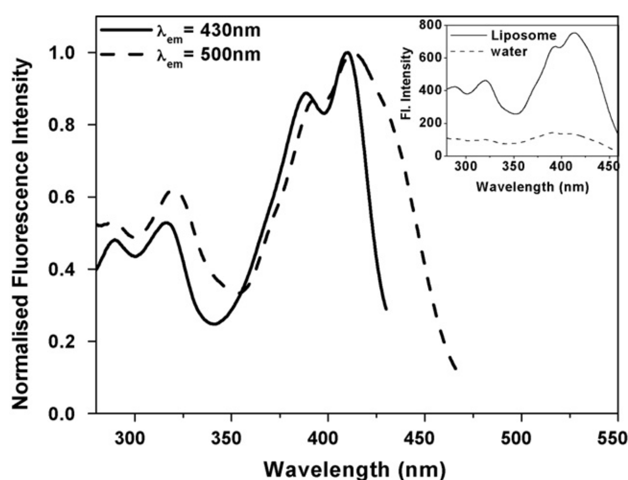

Fig. 2. Normalised excitation spectra of DMAPEPy in DMPC vesicles (solid line at λ_{em} = 430 nm and dashed line at λ_{em} = 500 nm). [DMAPEPy] = 1×10^{-7} M, [DMPC] = 4.5×10^{-4} M, temperature = 10 °C. The inset shows the comparison of excitation spectra, showing the relative intensities, as obtained in liposome and water (with 1% EtOH).

3.2. Determination of partition coefficient

The success of a fluorescent molecule as a membrane probe depends on the ease of its incorporation into the membrane. The partition coefficient value is an essential physico-chemical parameter, which is a measure of lipophilicity of the fluorescent molecule and provides information about the fraction of it associated with the lipid. The partition coefficient (K_p) value of any fluorescent probe between the aqueous and liposome medium can be evaluated by fluorescence spectroscopy as long as there is a difference in a fluorescence parameter of the partitioning molecule (e.g. quantum yield, fluorescence anisotropy or fluorescence lifetime) when in the aqueous solution and after incorporation in the membrane [28]. In case of DMAPEPy, since the emission band at 427 nm shows a gradual change in its intensity with the lipid concentration, it can be used as a parameter to determine the partition coefficient.

Fig. 4 shows the variation of fluorescence intensity at 427 nm (F) as a function of DMPC concentration (L) at three temperatures, 10 °C (solid gel phase), 23 °C (at the phase transition temperature) and at 35 °C (liquid crystalline phase). The fluorescence data show a hyperbolic dependence of F on L. The K_p values are calculated using the relation $F = F_0L/(55.6/K_p + L)$, where F is the fluorescence intensity at 427 nm at lipid concentration L, F_0 is the maximum flu-

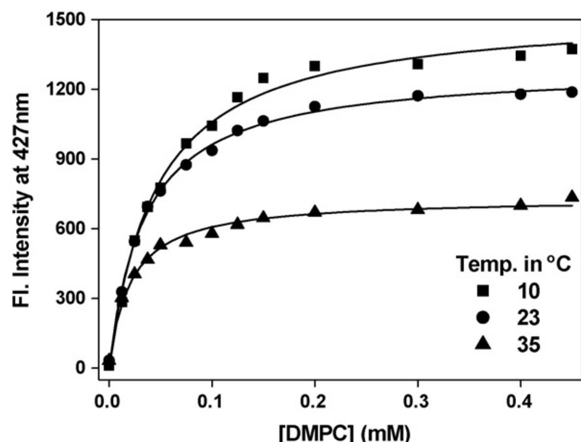

Fig. 4. Plot of fluorescence intensity of DMAPEPy at 427 nm (1×10^{-7} M) versus DMPC concentration at three temperatures 10 °C (solid gel phase), 23 °C (the phase transition temperature) and 35 °C (liquid crystalline phase). The solid lines represent the non-linear regression fits for the data according to the equation $F = F_0L/(55.6/K_p + L)$.

orescence resulting from total probe incorporation into membrane. The K_p values thus obtained are 1.23×10^6 (at 10 °C), 1.61×10^6 (at 23 °C) and 2.69×10^6 (at 35 °C). The K_p value increases with increase in temperature, which can be attributed to the increase in permeability of the membrane to DMAPEPy with increasing temperature. From the K_p value the lipid to probe ratio is calculated to be ≈2950 for [DMPC] = 2.5×10^{-4} M. This ratio was maintained for further work, where there will be maximum partitioning of DMAPEPy into the membrane and minimum interference from the fraction of the probe remaining in water. The fluorescence of the molecule in water being very weak it further minimises any possible spectral interference from the fraction of it remaining in water. Since the molecule shows reasonably high partition efficiency in both the phases of the bilayer, it is possible to work with submicromolar (10^{-7} M) concentration of the probe hence minimising the risk of perturbance to the membrane properties, which recommends it as a good membrane probe.

3.3. Quenching study

Fluorescence quenching studies on membrane bound fluorophore provide valuable information regarding their distribution and microenvironment. In the present study CPC has been used as a quencher. CPC is known to be an efficient quencher for pyrene and acts in a static mechanism [29]. Due to the presence of long hydrocarbon chain (C16) CPC easily gets incorporated into the bilayer membrane. The most likely position for the positive pyridinium moiety is near the negatively charged phosphate group of the phospholipid molecule. Thus it is expected that CPC when added at a concentration below its critical micelle concentration (CMC = 0.98 mM) [30] should selectively quench the fraction of probe located near the surface site.

Fig. 5A illustrates the emission spectra of DMAPEPy in DMPC unilamellar vesicles with varying amount of CPC. As is clear with the addition of CPC, the 499 nm band intensity decreases gradually and by 0.4 mM of CPC only the structured 427 nm peak exists. Thus the quenching study also supports the bimodal distribution of DMAPEPy in the liposome. Fig. 5B indicates the Stern-Volmer plot for the quenching of DMAPEPy in DMPC vesicles by CPC. The Stern-Volmer quenching constant is found to be 3.8×10^3 M^{-1}. This high value of K_{sv} supports the static nature of quenching of DMAPEPy by CPC in the bilayer.

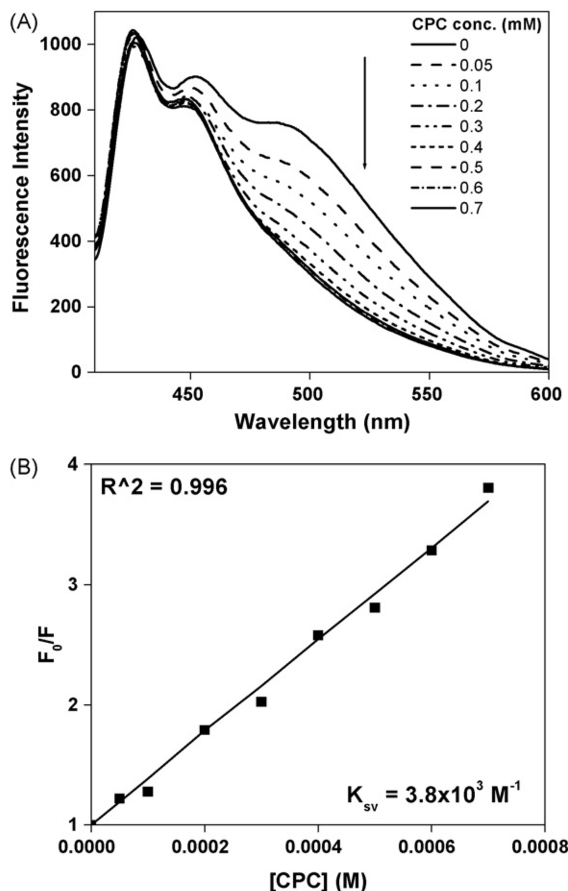

Fig. 5. (A) Emission spectra of DMAPEPy in DMPC unilamellar vesicles (0.25 mM) at varying amount of cetylpyridinium chloride. [CPC] = 0–0.7 mM, [DMAPEPy] = 1×10^{-7} M, temperature = 10 °C (solid gel phase). (B) Stern-Volmer plot for the quenching of DMAPEPy in DMPC vesicles by CPC.

3.4. Fluorescence lifetime studies

Fluorescence at both emission bands follows mono-exponential decay. In order to avoid spectral interference the lifetimes were measured at 425 and 540 nm by exciting the molecule at 370 nm. The lifetime at 425 nm is found to be 1.82 ns, which matches with that in *n*-hexane (1.85 ns) [26] and the lifetime at 540 nm is 3.00 ns similar to that in ethanol (3.05 ns) [26]. Table 1 summarises fluorescence lifetime data for DMAPEPy in DMPC unilamellar vesicles in the solid gel phase (15 °C) and liquid crystalline phase (35 °C). Though the change in lifetime with temperature is not very significant, the lifetime values reinforce the concept of bimodal distribution of DMAPEPy in the bilayer.

3.5. Temperature dependence membrane properties as probed by DMAPEPy

The response of a potential molecular probe to the phase transition is often taken as a convenient test for its applicability for

Table 1
Fluorescence lifetime data for DMAPEPy in DMPC unilamellar vesicles in the solid gel phase (15 °C) and liquid crystalline phase (35 °C)

Temperature (°C)	At 425 nm		At 540 nm	
	τ (ns)	χ^2	τ (ns)	χ^2
15	1.82	1.20	3.00	1.08
35	1.52	1.14	3.12	1.09

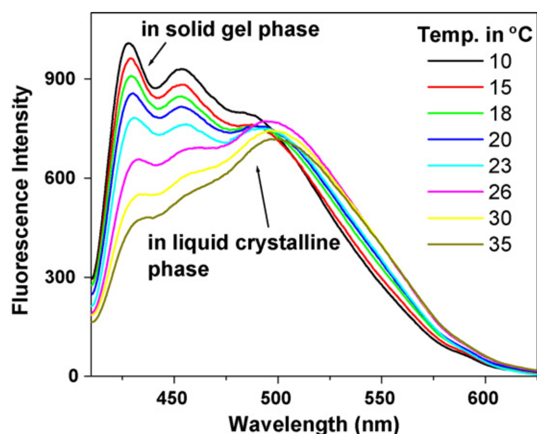

Fig. 6. Emission spectra of DMAPEPy in DMPC unilamellar vesicles with increasing temperature. [DMAPEPy] = 1×10^{-7} M, [DMPC] = 0.25 mM, λ_{ex} = 390 nm.

membrane studies. Fig. 6 shows the temperature dependence emission spectra of DMAPEPy in DMPC unilamellar vesicles. As is clear from the figure, there is a strong dependence of the spectral behaviour of the molecule on temperature. At low temperature, *i.e.* in the solid gel phase, the emission spectrum shows the structured bands at 427 nm but as the temperature increases the structured emission shows significant decrease in intensity and at high temperature, *i.e.* in the liquid crystalline phase, only the broad structureless band exists. There is a marked difference in the spectral behaviour of DMAPEPy in the two phases of the bilayer, which makes it feasible to identify the physical state of the lipid membrane just by visual inspection of the fluorescence spectrum. Such glaring difference in the spectral profiles with phase change in the membrane is not seen in any of the available membrane probes.

Since the molecule shows almost similar partition coefficient values in both solid gel and liquid crystalline phases of the membrane, the observed spectral changes with increasing temperature is due to the effect of temperature induced changes in the lipid matrix and not because of leaching out of the probe to the bulk aqueous medium unlike in case of Prodan [15].

The fluorescence intensity of the 427 nm band is found to be very sensitive to the temperature-induced changes in the lipid vesicles (Fig. 7A). There is a gradual change in fluorescence intensity with temperature showing the maximum change at 23 °C, the phase transition temperature of DMPC vesicle. Thus, the change in intensity can be considered as an index of thermotropic phase behaviour of the membrane and the emission intensity at 427 nm provides a convenient means to estimate the phase transition temperature in the bilayer.

The main phase transition or chain-melting transition in a lipid bilayer imparts fluidity to the membrane. In solid gel phase the bilayer is in a state of high rigidity and conformational order, which changes to a more fluid and disordered liquid crystalline phase above the phase transition temperature. Temperature dependence studies of DMAPEPy in viscous liquids such as glycerol and ethylene glycol have shown that its fluorescence anisotropy is very sensitive to the change in the medium viscosity [26]. In order to examine the response of fluorescence anisotropy of DMAPEPy to the change in viscosity in the lipid bilayer with temperature, temperature dependence experiments were carried out and fluorescence anisotropy was monitored at two emission maxima, 427 and 500 nm. Fig. 7B shows the variation of fluorescence anisotropy of DMAPEPy in DMPC unilamellar vesicles with increase in temperature at the two emission maxima, 427 and 500 nm. The anisotropy at both the wavelengths show very good sensitivity to the increased fluidity of the bilayer with temperature. The anisotropy value decreases

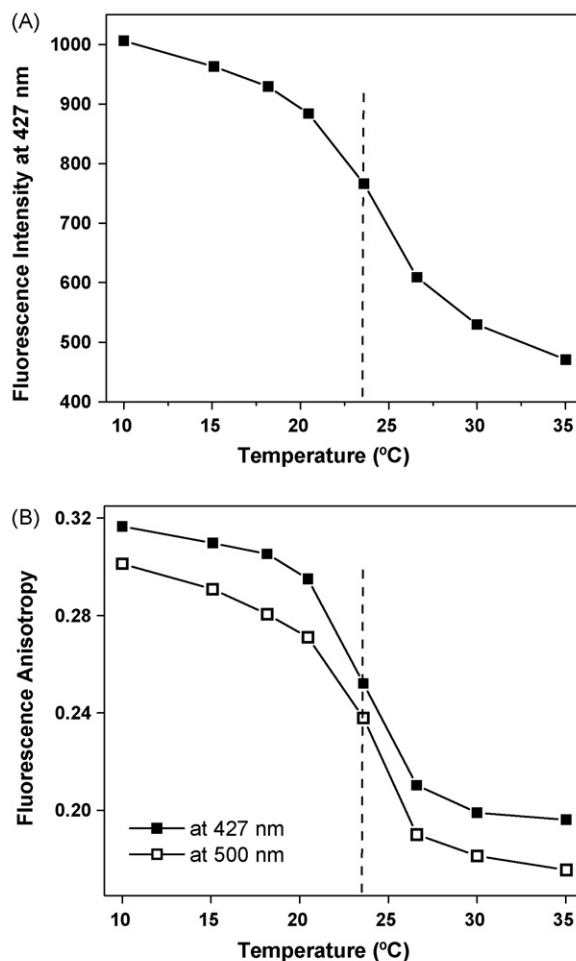

Fig. 7. Variation of (A) fluorescence intensity at 427 nm (λ_{ex} = 390 nm) and (B) steady-state fluorescence anisotropy (at λ_{ex} = 390 nm, λ_{em} = 427 nm and λ_{ex} = 430 nm, λ_{em} = 500 nm) of DMAPEPy in DMPC vesicles as a function of temperature.

gradually with temperature and the maximum change is observed at the phase transition temperature for both the emissions. Thus, DMAPEPy provides another independent fluorescence parameter in terms of its anisotropy for the study of membrane properties.

The observed spectral changes with increasing temperature is due to the redistribution of probe between the two sites of occupancy in the lipid matrix. If there were no effect of the non-radiative decay rate (k_{nr}) the intensity of the 500 nm fluorescence band, which is due to the probes located at polar site, should increase concomitantly with the decrease in the intensity of the 427 nm band. But earlier studies with solvents such as glycerol and ethylene glycol have shown that the emission intensity of DMAPEPy is very sensitive to change in viscosity [26]. The decrease in intensity at 427 nm with increase in temperature is due to decrease in both, the probe population at the non-polar site and in viscosity of the membrane. Whereas at 500 nm, the increase in intensity due to the increased population is offset by the decrease in intensity due to increased fluidity of the membrane and the intensity remained almost constant with temperature.

3.6. Sensitivity of DMAPEPy to the effect of cholesterol on DMPC bilayer

Incorporation of cholesterol brings about major changes in the properties of lipid vesicles. It has a very high affinity towards

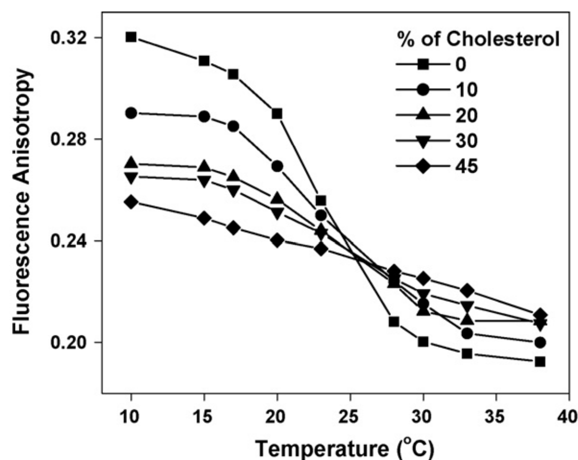

Fig. 8. Variation of fluorescence anisotropy of DMAPEPy at 427 nm with temperature at varying amount of cholesterol. [DMAPEPy] = 1×10^{-7} M, [DMPC] = 0.25 mM, [Cholesterol] = 0–45 mole% of DMPC, λ_{ex} = 390 nm and λ_{em} = 427 nm.

phospholipids and can be incorporated in concentrations as high as 1:1 or even 1:2 molar ratios of cholesterol to lipid in the membrane. Cholesterol reduces the enthalpy of phase change, thus with increasing addition of cholesterol the phase transition gradually gets blurred and is completely eliminated at high concentration when the enthalpy of phase change becomes zero [27]. Since DMAPEPy monitored successfully the temperature induced change in fluidity of the bilayer it is expected to monitor the changes induced by addition of cholesterol as well. The fluorescence anisotropy profiles show clearly the blurring of phase transition and by 45 mole% of cholesterol the complete elimination of the phase transition is observed (Fig. 8). The increase in the fluorescence anisotropy value of DMAPEPy in the liquid crystalline phase and the decrease of fluorescence anisotropy in the solid gel phase of DMPC with addition of higher mole% cholesterol show the effect of cholesterol in altering the fluidity of the membrane.

3.7. Response of DMAPEPy to vesicles of different phospholipids

The packing property of a phospholipid bilayer is dictated by the nature of the phospholipid that constitutes it, which on the other hand depends on the nature of the head group and lipid chain (length and presence of unsaturation) [3]. The response of DMAPEPy to the phase transition of vesicles constituted from different phospholipids has been examined. The phospholipids studied are DPPC and DPPG. The choice of DPPC is to increase the chain length from C14 in DMPC to C16 in DPPC and of DPPG is to change the charge on the head group (negatively charged, C16).

Fig. 9 depicts the emission spectra of DMAPEPy in (A) DPPC and (B) DPPG vesicles at three temperatures, *i.e.* at 30 °C (solid gel phase), 42 °C (phase transition temperature) and 52 °C (liquid crystalline phase). The increase in intensity of DMAPEPy is remarkable in both DPPC and DPPG liposome as compared to that in water, which shows its high affinity towards these vesicles. Thus, the partitioning of DMAPEPy is not limited by the negative charge on the head group of DPPG. In both the cases the emission spectra obtained are combination of a structured emission and a broad emission, similar to that obtained in DMPC vesicle. And the 427 nm band shows good response to the temperature-induced changes in the bilayer. The fluorescence anisotropy value is also found to respond well to the thermotropic changes in these vesicles. Thus, the sensitivity of DMAPEPy is not limited to neutral phospholipids and it can conveniently be employed for the study of all kinds of phospholipids.

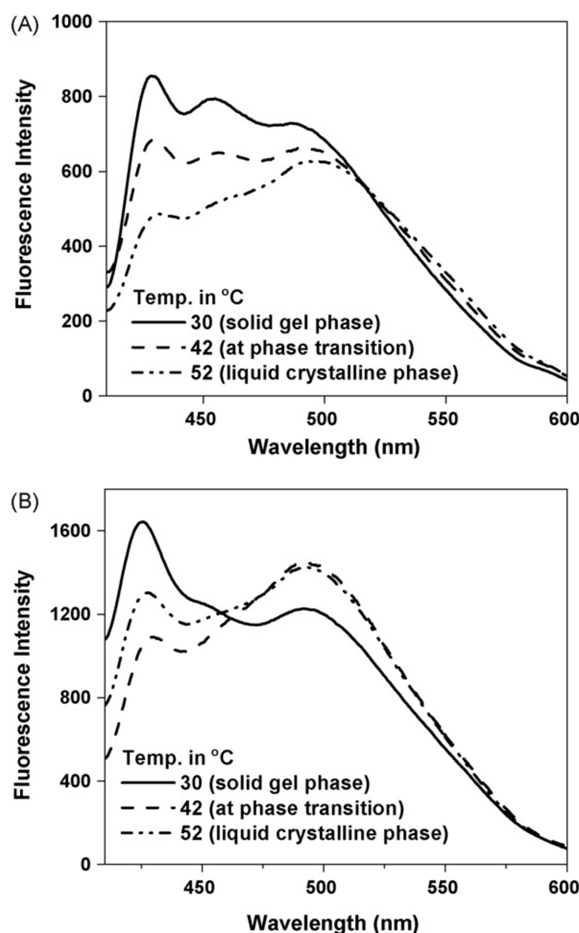

Fig. 9. Emission spectra of DMAPEPy in (A) DPPC and (B) DPPG vesicles (0.25 mM) at 30 °C (solid gel phase), 42 °C (at phase transition) and 52 °C (liquid crystalline phase), (λ_{ex} = 390 nm).

4. Conclusions

The present work proposes a proficient fluorescent molecular probe for the study of lipid bilayer membrane. DMAPEPy shows high affinity towards phospholipid vesicles in both solid gel as well as liquid crystalline phases. Fluorescence spectral behaviour, quenching studies and lifetime data indicated the bimodal nature of its distribution in the bilayer. The molecule reports on the polarity of the bilayer in terms of its fluorescence spectral position. The polarity parameters for the two sites of occupancy estimated suggest an isopropanol like environment and a non-polar hydrocarbon like environment for the probe in the bilayer. The spectral behaviour of the molecule shows spectacular response to the phase change in the bilayer and the fluorescence intensity of the 427 nm band can be used as a index of phase change in the bilayer. The steady state fluorescence anisotropy of DMAPEPy is equally sensitive to the phase change in the bilayer membrane. Fluorescence anisotropy at 427 nm as well as at 500 nm are found to be sensitive to the thermotropic changes in the bilayer properties. It is found to monitor successfully the changes induced by cholesterol in the bilayer. DMAPEPy offers potential tools in terms of its spectral position, fluorescence intensity and fluorescence anisotropy towards bilayer membrane studies and can be used both as a polarity sensitive probe as well as an anisotropy probe for the study of phospholipid vesicles. The spectacular difference in the emission spectra of DMAPEPy at the two phases of bilayer makes it unique in

the existing genre of fluorescent molecular probes for lipid bilayer membranes.

Acknowledgements

This research was partly supported by a research project funded by the Department of Science and Technology, Govt. of India. The authors thank SAIF, IIT Madras for access to fluorimeter and Prof. P. Ramamurthy and Mr. Kumaran of NCUFP, University of Madras for fluorescence lifetime measurements.

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