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Solvent relaxation in lipid bilayers with dansyl probes

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The solvent relaxation properties of the dansyl group attached to two lipids (dansylphosphatidylethanolamine and dansylphosphatidylserine), a fatty acid (dansylundecanoic acid), and two drugs (dansylbenzocaine and dansylpropranolol) were compared in a variety of different lipid systems. Several methods for characterising solvent relaxation were compared in detail for dansylpropranolol in bilayer vesicles of egg phosphatidylcholine. It was shown that the relaxation process is non-monoexponential; nevertheless, for comparative purposes, a model was adopted in which the lifetime associated with the negative exponent in a two exponential decay analysis, obtained at a particular energy on the red edge of emission, was taken as an approximation to a 'solvent relaxation' rate. A negative exponent, indicative of solvent relaxation processes, occurring in the nanosecond time-scale, was found only for dansylpropranolol, dansylPE and dansylundecanoic acid. On addition of the spin probe, 5-doxylstearate, the negative exponent was unaffected in liquid-crystalline phase lipids but was no longer found in gel-phase lipid in the case of dansylpropranolol, while for dansylPE the relaxation time was reduced. On the basis of these types of measurement it was possible to distinguish between different lipid environments using the same probe or between different dansyl environments of the different probes in the same lipid in cases where this would have been difficult or impossible solely on the basis of steady-state or fluorescence lifetime measurements.

Introduction

Fluorescence techniques have been widely used to probe the motional, structural and binding properties of membrane lipids and proteins. Much information on the motion of a fluorescence probe, and by inference its environment, may be gained from steady-state fluorescence emission and polarization measurements. The use of time-resolved techniques, however, enables fluorescence lifetime and anisotropy decay measurements to be made, providing much further information on the probe environment and dynamics. Time-resolved emission spectra can also be obtained enabling changes in the emission specta occurring during the fluorescence lifetime to be monitored. These changes can be indicative of excited-state reactions or solvent relaxation processes.

The solvent relaxation process occurs when the dipole moment of an excited molecule is sufficiently different from that of the ground state. In a polar environment, the solvent cage will then relax by dipole orientation interactions to accommod-

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Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; egg-PC, egg-yolk phosphatidylcholine; egg-PE, egg-yolk phosphatidylethanolamine; PS, phosphatidylserine; PE, phosphatidylethanolamine.

ate, more suitably, the changed dipole moment. The initially excited state of the fluorophor converted into a second excited species with a rate constant K_R. The fluorescence decay process of the system on the extreme red edge of emission can be simply described as:

$$F(t) = \sum_{i=1}^{m} \alpha_i e^{-t/\tau_{F_i}} - \sum_{j=1}^{n} \beta_j e^{-t/\tau_{R_j}} \quad (1)$$

where the first term indicates components with lifetimes τ_{F_i}; α_i, indicating the fractional contribution to $F(t)$. The minus in the second term indicates solvent relaxation (or another excited-state process) with a decay constant(s) τ_R $(= 1/K_R)$ and fractional contribution β_j. The negative exponent indicates the relaxation process (i.e., red shift), which may not be a simple exponental process. Examples of the types of molecule showing this type of process in membranes or proteins include 2-toluidinylnaphthalene-6-sulphonic acid [1–4], tryptophan [5–7] and 5-dimethylaminonaphthalene-1-sulphonyl (dansyl, [8]). Previously, the solvent relaxation properties of dansylPE were reported [8], and in this work we have extended these observations to the examination of a range of dansyl probes using τ_R to describe the solvent relaxation as defined in Eqn. 1. Although the term is an approximation of a more complex process, its usefulness in the study of lipid bilayer systems has been demonstrated.

The solvent relaxation properties of dansyl conjugated to propranolol have been examined in a wide variety of lipid systems and compared with analogues of a fatty acid, dansylundecanoic acid, an anesthetic, dansylbenzocaine, and two lipids, dansylPE and dansylPS. Dansylpropranolol has been shown to interact with membrane lipids [9,10] and with the lipid/protein interface membrane proteins, these two binding sites being distinguishable using steady-state fluorescence techniques [10]. The results of the present study indicate that although the respective environments of the various dansyl groups in lipid bilayers are indistinguishable using steady-state fluorescence methods, they can be resolved using time-resolved fluorescence measurements of the solvent relaxation process.

Materials and Methods

Dansylpropranolol was obtained from Calbiochem; dansylPE, dansylPS and dansylundecanoic acid were from Molecular Probes and dansylbenzocaine was a gift from E.K. Rooney. Egg-PC and egg-PE were from Lipid Products (Nutfield Nurseries, Surrey, U.K.); other lipids were from Sigma. Spin-labeled fatty acids were from Aldrich. Fluorophores and lipids were kept in chloroform or methanol, as appropriate, under nitrogen at −20°C.

Liposomes

Multilamellar liposomes were made by removing solvent from a mixture of probe and lipid in a tube with a stream of nitrogen gas. Phosphate buffer (0.1 M, pH 7.4) was then added and the contents of the tube were vortexed vigorously. In the case of DMPC and DPPC liposomes the temperature was about 10 deg. C above the respective phase transitions.

For fluorescence measurements, samples were placed in a thermostated cuvette holder and excited with 5 MHz, 200 ps (full-width half-maximum) pulses of 300 nm light from an argon-ion-laser driven (Coherent) cavity dumped, rhodamine 6G dye laser and frequency-doubling crystal. Emission, spectrally dispersed by a monochromator was examined using single-photon counting electronics and analysed by non-linear least-squares methods using on-line computer facilities. The deconvolution procedures have been described elsewhere [11].

Time-resolved emission spectra were obtained in one or two ways. In the first, the upper upper and lower discriminators on a multichannel analyser were set at voltages V_1 and V_2, corresponding to times t_1, and t_2 in the time-to-amplitude converter. This set a gate width $\delta t = t_2 - t_1$ which could be moved to different times, T, across the decay profile, collecting photons only within the discrete time slice. Then, for selected times T, the wavelength was varied on the monochromator by a motorized wavelength controller synchronized with the multichannel analyser in multichannel scale mode, and the time-resolved emission spectra were obtained thereby (Fig. 1). In this method the measured decay, $I(t)$, is a convolution

of the true decay, $G(t)$, whith the pulse or instrument response function, $G(t)$ where:

$$I(t) = \int_0^t P(t')G(t-t')\mathrm{d}t' \tag{2}$$

Thus, some distortion in the time-resolved emission spectra may occur; although at very early times, with narrow gates, the excitation corresponds to a δ function and at late times is negligible [8].

A second method [1,12] obtains $I(t)$ at regular intervals of λ (5–10 nm) and $G(t)$ is obtained by deconvolution [11].

Then time-resolved emission spectra, $Y(\lambda, t)$ are created by:

$$Y(\lambda,t) = I(\lambda)\cdot G(\lambda,t)/A \tag{3}$$

where $I(\lambda)$ is the intensity of the total fluorescence spectrum (obtained separately), $G(\lambda,t)$ is the deconvolved decay at wavelength λ and A is the area of the decay at λ. Spectral resolution is set by the number of decays obtained and time-resolution is limited only by the channel width in the multichannel analyser. In this instance only an accurate representation of $I(t)$ was required (as judged by the reduced χ^2, [11] and so $G(t)$ is chosen as:

$$G(t) = \sum_{i=1}^{10} A_i e^{-t/\tau_i} \tag{4}$$

where τ_i are fixed and A_i varied [13] as described previously [12]. Once the general nature of the solvent relaxation process had been examined by obtaining time-resolved emission spectra, it then remained to obtain a parameter describing the rate of relaxation of the environment around the excited state of the molecule in question. In this work we compared four different approaches:

(i) The simplest method for obtaining a quantitative solvent relaxation function parameter is to assume a single exponent relaxation process and fit the decay of the fluorescence to Eqn. 1 letting $n = 1$. The assumption made is that there are two states, an initial Franck Condon excited state and a completely relaxed state, and that the relaxation is described by a single rate constant. Generally, for the dansyl probes, Eqn. 1 gave a reasonable fit to the data when both n and m were set to 1.

(ii) A solvent relaxation function, again under a two-state relaxation assumption, may also be obtained from [3,4]:

$$\tau_R = \left(\frac{\upsilon_0 - \upsilon_{cg}}{\upsilon_{cg} - \upsilon_\infty}\right)\tau_F \tag{5}$$

where τ_F is the fluorescence lifetime, and υ_0, υ_{cg}, and υ_∞ the centres of gravity of the emission spectra for the initial Frank Condon excited state, the total fluorescence spectra and the fully relaxed state, respectively. υ_0 and υ_∞ correspond to the early- and late-gated time-resolved emission spectra described above.

(iii) If time-resolved emission spectra are analysed according to the model of Bakhshiev [14] then, in the notation of Brand and co-workers [2], the decay of the sample at energy υ (cm^{-1}), $I(\upsilon, t)$ is represented as the product of an electronic decay term, $i(t)$, and spectral shift term $\delta(\upsilon, t)$:

$$I(\upsilon, t) = i(t)\delta(\upsilon, t) \tag{6}$$

The spectral shift term may be obtained from time-resolved emission spectra [15,2]; in this work we obtained the term from the time-resolved emission spectra (see, for example, Fig. 2) obtained by the deconvolution method described above.

(iv) Another approach is to divide the $I(\upsilon, t)$ taken at the red edge of emission by the total fluorescence decay $F(t)$ (over all υ), assuming that the kinetics of the decay of fluorescence are independent of the wavelength and that the $F(t)$ does not contain the spectral relaxation (i.e. $F(t) \equiv i(t)$):

$$\delta(\upsilon, t) = I(\upsilon, t)/F(t) \tag{7}$$

The results of methods (iii) and (iv) give very similar results. Fig. 3 illustrates a result obtained by method (iv) and it can be seen that the relaxation process is complex (non-monoexponential) and that a single parameter, such as τ_R above, can be only an approximation. Nevertheless, for the

purpose of examining the behaviour of similar fluorophors in varying environmental conditions, such as for dansyl probes in model lipid membranes as examined here, analysis using the two-state model was considered appropriate. This of necessity limits interpretations pertaining to the precise mechanism of the solvent relaxation process around the dansyl probes. For comparative purposes it was chosen to analyse the decay of the emission at 575 nm according to Eqn. 1. This assumes that the fluorescence decay itself is a mono-exponential process. In some cases, however, some slight improvement of the fit of the data could be made using a three-exponent decay model. The improvement was not particularly consistent, however, and often the third exponent showed a short lifetime which was difficult to distinguish from that expected from light scattering. Thus, for comparative purposes in the semi-empirical manner intended here, we chose to interpret our data in terms of the two-exponent decay.

Results

Time-resolved emission spectra for dansylpropranolol in egg-PC vesicles are shown in Figs. 1 and 2. Fig. 1 illustrates early- and late-gated spectra (obtained by setting a gate width δt, which is the first of the two methods described in Materials and Methods), where the spectrum obtained 50 ns after excitation is shifted in wavelength by $15 \cdot 10^{-2}$ cm^{-1} (67 nm) towards the red. Taking the centres of gravity of the early, late and total

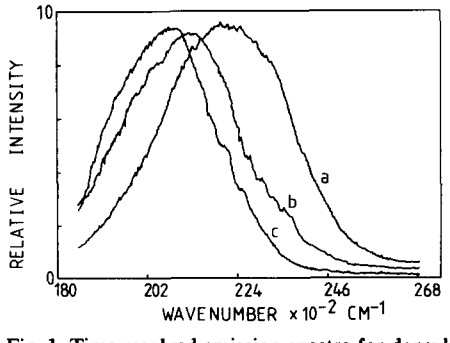


Fig. 1. Time-resolved emission spectra for dansylpropranolol in egg-PC liposomes at 25°C corresponding to the initial excited state (a) obtained by restricting observation to 0–2 ns after excitation, the total emission (b) and the fully relaxed state (c), obtained in the 30–50 ns region.

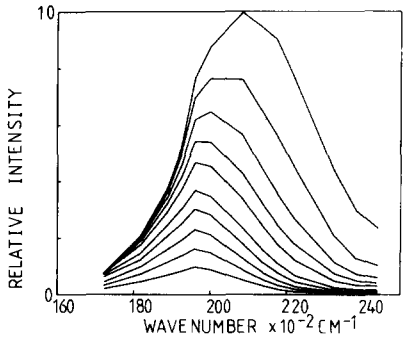


Fig. 2. Time-resolved emission spectra reconstructed from fluorescence decays taken a disscrete wavelengths in a stepwise manner to dansylpropranolol in egg-PC liposomes (25°C) for different times after the excitation pulse. As the time of observation is moved further in time from the time of excitation then the spectral emission maxima moves correspondingly towards the red end of the spectrum (left) and has the expected lower energy of emission. The extremes correspond to the initial (unrelaxed) Frank Condon state and fully relaxed state.

fluorescence spectra and applying Eqn. 5 (see (ii) in Materials and Methods) a value for the solvent relaxation function can be obtained. However, for comparative purposes we found it more convenient in this work to use the lifetime associated with the negative pre-amplitude in a two-component analysis of the emission at the red edge (see (i), in Materials and Methods), the results of which are detailed below. In Fig. 2, the time-resolved emission spectra for dansylpropranolol in egg-PC are shown, constructed using the second method described in Materials and Methods, collecting decays (such as those illustrated for dansylpropranolol in Fig. 4) at regular wavelength intervals. The red shift and energy decrease at times taken further from excitation is clearly shown.

The data used to obtain the reconstructed time-resolved emission spectra can also be used to obtain a solvent relaxation function (see (iii) in Materials and Methods), as illustrated in Fig. 3. The fact that the relaxation process is non-mono-exponential is clearly illustrated, since the intensity is on a log scale, so that a single exponential would be expected to show as a linear plot. Similar results showing a non-monoexponential relaxation process has been shown for 2-*p*-toluidinonaphthalene-6-sulfonate in egg-PC vesicles [1], while the results for the probe bound to apomyoglobin [2] showed that two relaxation con-

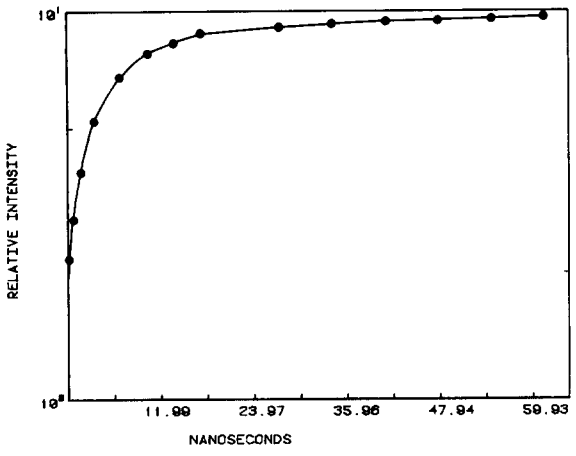
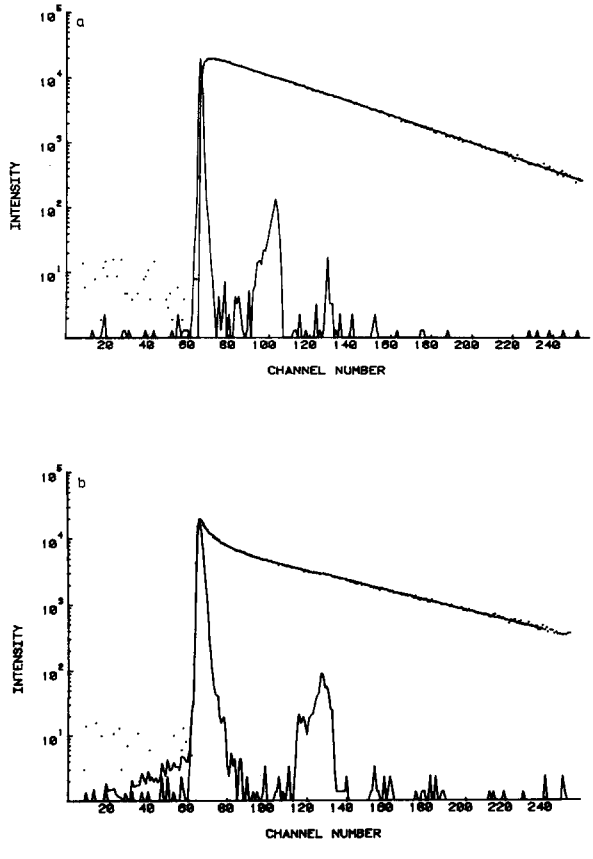


Fig. 3. The solvent relaxation function shown as an increase in intensity with time obtained from the same data used to obtain the reconstructed time-resolved emission spectra. In essence, the data such as are illustrated in Fig. 4(a), containing contributions from both τ_F and τ_R (Eqn. 1), as well as the excitation pulse with which it is convoluted, are processed so as to remove τ_F and deconvoluted to 'remove' the excitation pulse, leaving τ_R, the apparent relaxation time which is a reciprocal of the solvent relaxation rate constant.



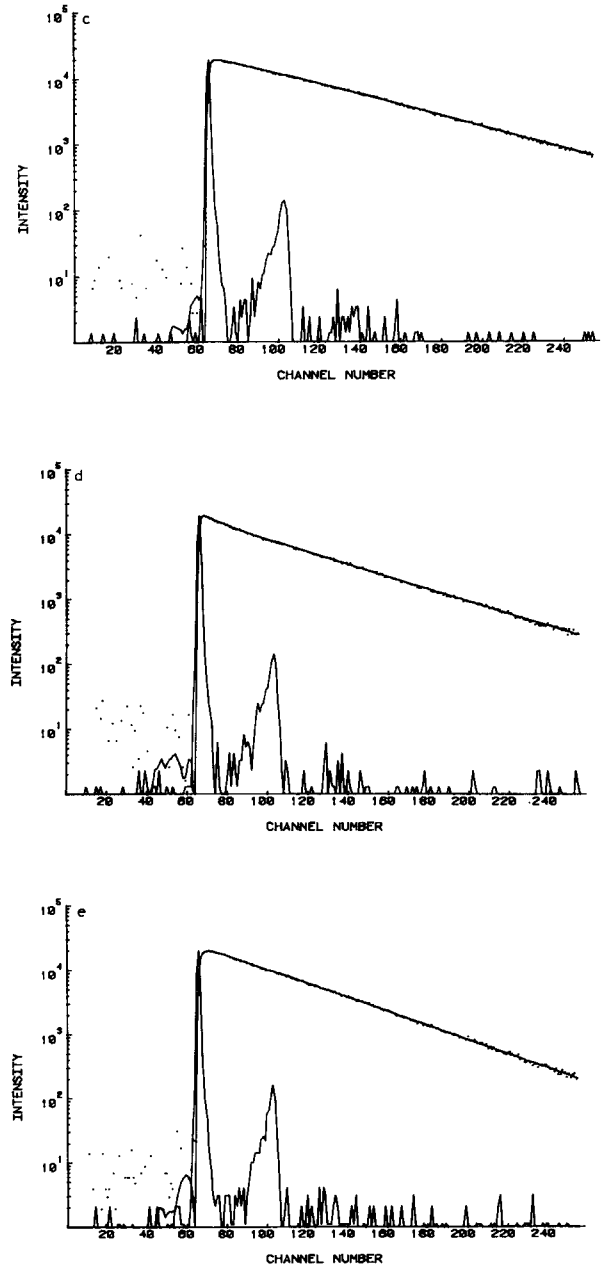


Fig. 4. Fluorescence decays of: (a), dansylpropranolol in DPPC above the phase transition at 575 nm. The initial 'rise' in intensity characteristic of solvent relaxation is clearly apparent and a two-exponential fit to the data reveals a negative exponent (see Table I); (b) at 470 nm no relaxation is seen, as expected; (c) below the phase transition at 575 nm as for (a); (d) below the phase transition, plus 5-doxylstearate (575 nm) the initial rise is no longer apparent and no negative pre-exponent in a two exponential analysis is found; (e) the same sample taken above the phase transition – the negative pre-exponent re-appears (see Table I).

stants could be recovered. Bearing in mind the difficulties in adequately describing the relaxation process, we used the two-state relaxation model (see (i) in Materials and Methods) in order to compare both the same dansyl probe in different lipid systems and a variety of molecules containing the dansyl group.

Fluorescence decays taken at the red edge of emission for dansylpropranolol in DPPC liposomes at temperatures above and below the phase transition temperature and a decay taken at the blue edge are shown in Fig. 4. The presence of a relaxation process is easily diagnosed when the fluorescence decay profile shows a slight rise before the decay predominates. A least-squares analysing program [11] was used to recover best-fit parameters and negative pre-exponents from decays which showed the initial rise characteristic. In all cases a monoexponential fit was inadequate, while in systems showing a solvent relaxation characteristics Eqn. 1 (m, $n = 1$) gave a reasonable fit to the data. Often further improvements to the fit were obtained if a three-component decay was assumed, but usually the lifetime associated with the negative pre-exponent was little affected. The rise-time associated with the relaxation process

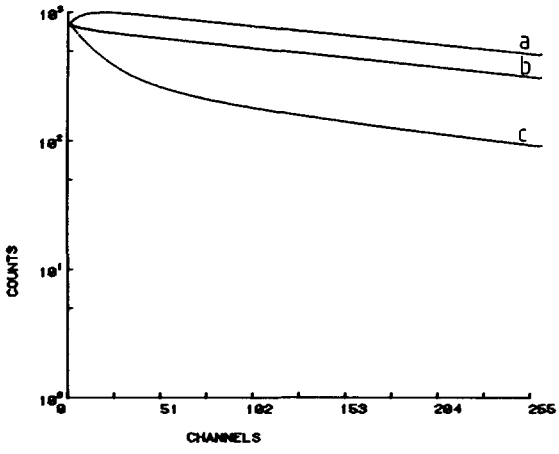


Fig. 5. Deconvolved fluorescence decays (0.05 ns/channel) of dansylpropranolol in egg-PC bilayers at 25°C for (a) 570 nm; (b) 520 nm (c) 470 nm.

and recoverable as the lifetime component associated with the negative pre-exponent (τ_R) is more clearly seen in deconvolved decays as illustrated in Fig. 5. Comparisons of τ_R values for dansylpropranolol in various lipid bilayers are documented in Table I. For DMPC and DPPC liposomes the τ_R values for the gel phase were

TABLE I

TWO-COMPONENT ANALYSIS OF THE FLUORESCENCE DECAY OF DANSYLPROPRANOLOL IN VARIOUS LIPIDS AT 575 nm

Differences greater than 0.3 ns in τ are significant.

	Temp.	EM_{max} [a]	A_1 [b]	τ_1	A_2	τ_2	χ^2 [c]
Egg-PC	25	525	−0.35	2.01	+1.80	14.28	1.32
DPPC	25	517	−0.40	2.80	+1.75	17.34	1.32
	50	527	−0.94	0.88	+2.27	13.96	1.68
DMPC	10	508	−0.38	3.68	+1.64	19.60	1.63
	35	523	−0.75	1.39	+1.83	14.07	1.49
DOPC	25	522	−0.71	2.02	+2.90	13.36	1.17
PS	25	520	−0.59	2.69	+1.77	15.58	1.33
Egg-PE	25	525	−0.50	1.99	+1.95	11.89	1.38
Egg-PC/cholesterol [e]	25	523	−0.52	2.44	+1.70	13.84	1.28
DPPC + 5-doxylstearate	25	517	+0.46 [d]	4.48	+1.51	15.58	1.63
	50	517	−0.80	0.76	+2.12	13.16	1.82

[a] Steady-state fluorescence emission maxima.
[b] Pre-exponent, negative sign indicates τ_1, is the solvent relaxation function (τ_R).
[c] Reduced chi-square.
[d] Positive pre-exponent and therefore no solvent relaxation.
[e] 1:0.5 molar ratio.

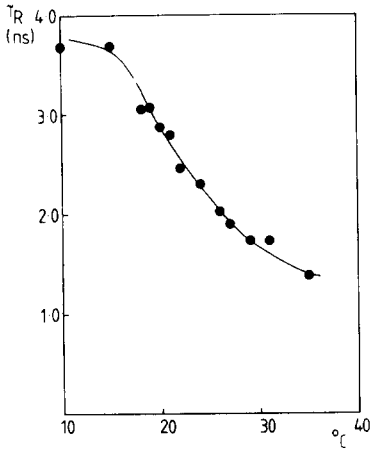


Fig. 6. Effect of temperature on τ_R for dansylpropranolol in DMPC vesicles.

significantly greater than the respective values above the phase transition. A plot τ_R against temperature is shown in Fig. 6. Since the τ_R values relate more to motion in the glycerol backbone region of the bilayer appear, they are less sensitive to the phase transition than are probes of the acyl chain region [16]. The emission maxima shift to a longer wavelength (Table I) when dipalmitoyl and dimyristoyl liposomes are taken above the phase transition, but egg-PC (above the phase transition) also shows a red shift with increasing temperature. This means that comparisons between lipids in the same phase but at different temperature cannot easily be made on the basis of emission maxima. The solvent relaxation time is also temperature-sensitive and the same difficulty is encountered in comparing different lipids. The effect of decreasing the temperature below the phase-transition temperature and the effect of the addition of cholesterol were to increase τ_R. Compared to egg-PC, PS had a long relaxation time. These results, are at least in reasonable accord with previously documented results for probes of the motion of the acyl chain region such as diphenylhexatriene [16].

The effect of adding spin-labelled fatty acids was examined in the first instance to confirm that the location of the dansyl moiety was in the glycerol backbone region and not deeper in the hydrophobic region of the bilayer. Evidence for the location in the former region is shown in Fig. 7, where the quenching effect of 5-doxylstearate (lipid/dansylpropranolol/5-doxylstearate, 100 : 1 :1, molar ratio) is shown to be greater than 16-doxylstearate, with the nitroxide radical at the centre of the bilayer. In DPPC liposomes above the phase transition (Fig. 4), no effect on τ_R was found as for egg-PC (both above the phase transition). In contrast, the two-component analysis of the fluorescence decay of dansylpropranolol in DPPC liposomes below the phase transition did not reveal a lifetime component with a negative amplitude (Fig. 4 and Table I), although at lower 5-doxylstearate concentrations (Lipid/ dansylpropranolol/5-doxylstearate, 100 : 1 : 0.2; molar ratio) it could again be recovered. When the same sample, not showing relaxation, was taken repeatedly through the phase transition τ_R was recoverable only in the liquid-crystalline phase. A second lifetime component of around 4 ns was found in the gel-state lipids. The emission maxima (see Fig. 7) were not effected by 5-doxylstearate. The effect of 5-doxylstearate on dansylPE in DPPC liposomes was a decreased τ_R (Table II), again with no effect above the phase transition. For dansylundecanoic acid only a marginal effect was found. To examine whether the effect could be mimicked by adding a free fatty acid, oleic acid was added under the same experimentnal conditions; no effect was observed, indicating that the effects of the spin probes on τ_R were attributable in some way to the nitroxide group.

Comparison of different dansyl probes

In Table II the solvent relaxation function τ_R

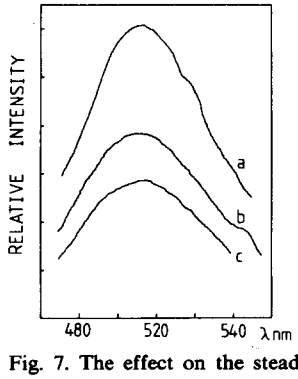


Fig. 7. The effect on the steady-state fluorescence spectra of dansylpropranolol in liposomes (a) control; (b) plus 16-doxylstearate; (c) plus 5-doxylstearate.

TABLE II

LIFETIMES (ns) ASSOCIATED WITH NEGATIVE PRE-EXPONENT FOR DIFFERENT DANSYL PROBES IN VARIOUS LIPID SYSTEMS

n.f., no negative pre-exponent found. n.d., not determined. 5-DS, 5-doxylstearate.

	Egg-PC 25°C	DPPC			
		10°C		50°C	
5-DS		−	+	−	+
Dansylpropranolol	2.01	2.80	n.f.	0.88	0.76
DansylPE	1.48	1.38	0.63	0.48	0.68
DansylPS	n.f.	n.f.	n.d.	n.d.	n.d.
Dansylundecanoic acid	n.d.	3.38	3.66	n.d.	n.d.
Dansylbenzocaine	n.f.	n.f.	n.d.	n.d.	n.d.

for the different dansyl probes in DPPC liposomes below the phase transition temperature are compared. In the absence of spin probe quenching, neither dansylbenzocaine not dansylPS showed a relaxation time (no negative pre-exponent), indicating either that the dansyl environment of dansylbenzocaine relaxes very quickly and is located more towards, or possibly at, the aqueous interface, or is located entirely in the hydrophobic region, which is unlikely since the emission spectrum is characteristic of a relatively polar environment. The relaxation times for the other probes was in the order dansylPE < dansylpropranolol < dansylundecanoic acid again, probably a reflection that a deeper location in the membrane was being probed, a more hydrophobic environment resulting in a longer τ_R. Examination of the steady-state emission maxima (Table I) alone was not sufficient to distinguish between the dansyl probes.

Discussion

In this work the emphasis has been on showing how the fluorescence properties of the dansyl group change when it is attached to different molecules in a membrane lipid bilayer. In fluorescence measurements the fluorophore is usually used as a 'probe' molecule and information about its physical characteristics is interpreted in terms of the characteristics of the surrounding molecules in the matrix of interest, i.e., the membrane, protein complex, etc. It is thus an indirect technique. In contrast, the solvent relaxation function of the type extracted from the measurements made in this work is a property of the molecular system of interest and not of the probe molecule.

A further useful property of solvent relaxation is that it occurs only when the altered dipole moment of the excited fluorophore results in a reorientation of the dipoles of the surrounding polar molecules, non-polar molecules in the environment of the fluorophore do not contribute. For membranes, since solvent relaxation in the aqueous region is extremely rapid, the region under examination is restricted to the membrane/water interface and the phospholipid headgroup region. This region is of importance in many membrane-related phenomena.

In the primary identification of solvent relaxation or other excited-state reactions the best method is probably to obtain time-resolved emission spectra. The most straightforward technique is to compare time-resolved emission spectra for time windows corresponding to early and late times in the fluorescence decay (early- and late-gated spectra). However, since the excitation pulse is still convoluted with the intensity decay, $I(t)$, taken for the particular time slices and wavelengths, some distortion of the spectra is unavoidable. Also, problems of uneven photomultiplier response at different wavelengths have to be considered. We therefore feel that this method is most useful as a diagnostic technique. The second method for obtaining time-resolved emission spectra (see Materials and Methods), which has been discussed in detail elsewhere [12], particularly as compared to the first method, also suffers from a number of drawbacks. These include, again, uneven photomultiplier response and also fairly involved computational analysis, apart from the long data-collection times required (several hours with the system described here; much longer with flash-lamp excitation). Regarding the analysis of the data in terms of a solvent relaxation function, we compared four approaches. The simplest method is the analysis of the emission at the extreme red edge of emission (see (i) in Materials and Methods). This has the advantage of rapid measurement (approx. 5 min), which may be important in examining unstable systems or short-lived states where rapid

data collection is a prerequisite. The finding of a negative amplitude in the two component analysis is in itself a fairly reliable indication of an excited state reaction or relaxation process. The second method (ii), above, was also investigated and was found to yield results similar to those from the red-edge analysis. However, time-resolved emission spectra had first to be obtained so that the advantage of the short data collection time of the formed method was lost. Both of these methods ascribe a single solvent relaxation function to a process which is clearly more complex. This was illustrated in the methods, (iii) and (iv), which yield the type of result illustrated in Fig. 3, and previously seen in other systems [1,2]. Thus, if the nature of the relaxation process could be simply ascribed, e.g., as a two-component system [2], then the latter methods would be preferred. In the case of dansylated molecules in the lipid systems, we felt that the red edge analysis provided a reasonable approach, at least for the comparative purposes we had in mind.

The results of comparison of dansylpropranolol in various lipid systems indicated that major differences in the nature of the lipid headgroup, fatty acyl chain length or degree of unsaturation do not result in substantial differences in τ_R. Similar conclusions have been reached in studies using 2-*p*-toluidinylnapththalene-6-sulphonic acid [3,4]. Nevertheless, τ_R was longer in gel phase lipids and in the presence of cholesterol. To some extent, comparisons between different systems at different temperatures are complicated by the sensitivity of τ_R itself to temperature.

In comparison with the different τ_R values obtained for various lipids, the effect of addition of the spin-labeled fatty acids were fairly dramatic. Thus, with dansylpropranolol in gel-phase lipids, a negative pre-exponent was no longer found in a multiexponential analysis of the red edge emission. Either τ_R became very rapid or the nature of the dansyl group environment had changed so that dipole-dipole relaxation was not occurrring. Whatever is the nature of the effect of the spin-labeled fatty acids, two factors are of interest. Firstly, only relatively low spin-label concentrations were required to produce the effect and secondly, the process was fully reversible with changing temperature. In contrast the effect with dansylPE was to lower τ_R in gel-phase lipids. The difference in the effects may reflect differences in the abilities of dansylPE and dansylpropranolol to form 'domains' or 'clusters' with the spin-labeled fatty acid. Although the nature of the effect remains to be explained, it may be useful in studying the effect of addition of perturbent molecules (drugs, etc.), to membranes.

On comparison of the different dansyl probes it was at first somewhat surprising that although the steady-state emission spectra were all very similar, the solvent-relaxation properties varied widely. In particular for dansylbenzocaine and dansylPS τ_R could not be recovered. For dansylbenzocaine the reason may be the relatively hydrophilic nature of the molecule resulting in a location for the dansyl group close to the lipid/water interface, where relaxation will be fast. Similarly, for dansylPS, the carboxyl group may ensure a relatively polar environment for the dansyl group. Thus, the nature of any charged species at the aqueous/headgroup region interface is clearly of importance and this factor certainly warrants further study. It should be noted that, in contrast to dansylPE, τ_R was recoverable for dansylpropranolol in PS bilayers so that it is not the charge at the bilayer surface itself that is important but rather how the charge on the dansyl-containing model determines the possible location of the dansyl group. The longest relaxation time we observed was for dansyl undecanoic acid, the dansyl group may be located more toward the hydrophobic centre of the bilayer than for any other of the probes.

In summary, we have compared solvent relaxation properties for different dansyl containing molecules in various lipid systems. Using the methods described, it was possible to distinguish between the various systems indicating the potential of time-resolved fluorescence methods in the study of biological membranes.

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