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A Model for the Interaction of 6-Lauroyl-2-(*N,N*-dimethylamino)naphthalene with Lipid Environments: Implications for Spectral Properties

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ABSTRACT

Although 6-lauroyl-2-(*N,N*-dimethylamino)naphthalene (LAURDAN) is now widely used as a probe for lipid systems, most studies focus on the effect of the lipid environment on its emission properties but not on the excitation properties. The present study is intended to investigate the excitation properties of LAURDAN in diverse lipid environments. To this end, the fluorescence properties of LAURDAN were studied in synthetic ester and ether phosphatidylcholines and sphingomyelin vesicles below, at and above the corresponding lipid main phase-transition temperature. The excitation spectra of LAURDAN in these environments always show at least two well-resolved bands. In the different lipid vesicles the behavior of the red band in the LAURDAN excitation spectra is sensitive to the lipid chemical environment near the probe fluorescent moiety and to the packing of the different lipid phases (gel and liquid crystalline). We propose that the interaction between the LAURDAN dimethylamino group and the ester linkage of ester phospholipids is responsible for the strong stabilization of LAURDAN's red excitation band in the gel phase of ester phospholipid vesicles. We discuss the consequence of these proposed ground-state interactions on LAURDAN's emission generalized polarization function. In the context of variable excitation wavelengths, information concerning solvent dipolar relaxation through excitation generalized polarization function is also discussed.

INTRODUCTION

Several naphthalene derivatives belong to the family of polarity-sensitive fluorescent probes, firstly designed and synthesized by Gregorio Weber for the study of the phenomenon of dipolar relaxation of fluorophores in solvents, bound

to proteins and associated with lipids (1–3). This family of probes includes 6-propionyl-2-(*N,N*-dimethylamino)naphthalene (PRODAN),† 6-lauroyl-2-(*N,N*-dimethylamino)naphthalene (LAURDAN) and 2'-(*N,N*-dimethylamino)-6-naphthoyl-4-*trans*-cyclohexanoic acid (DANCA) (Fig. 1). These probes all possess large excited-state dipoles. In polar solvents, and when the molecular dynamics of solvent dipoles occur on the time scale of the probe's fluorescence lifetime, a reorientation of solvent dipoles around the probe excited-state dipole may occur. The energy required for this reorientation results in a red shift of the fluorescence emission spectrum. Also, the excitation spectra of these naphthalene derivatives show spectral shape changes in solvents capable of establishing specific interactions such as hydrogen bonds (4). For example, in nonpolar solvents LAURDAN's excitation spectrum shows a nonresolved excitation band in the 300–420 nm wavelength range with a maximum located at 340 nm (4). In alcoholic solvents for example, LAURDAN's excitation spectra show a maximum at 370 nm and display a second excitation band with a maximum at about 390 nm (4). This second excitation band is due to a charge transfer state that is stabilized by solvent dipoles (ground-state $L\alpha$ conformation) (4).

In ester phospholipid vesicles, LAURDAN's emission and excitation spectra are sensitive to the packing of lipid molecules and consequently to their phase state. When the main lipid phase transition occurs, LAURDAN's emission spectrum shows a continuous red shift with no isoemissive point, and the position of LAURDAN's excitation maximum changes (5). In ester phospholipid vesicles in the gel phase, LAURDAN emission maximum is near 440 nm while in the liquid-crystalline state it is near 490 nm. During the phospholipid phase transition a continuous red shift of LAURDAN's emission spectrum is observed (4). This red shift was attributed to the reorientation of water molecules

†Abbreviations: DANCA, 2'-(*N,N*-dimethylamino)-6-naphthoyl-4-*trans*-cyclohexanoic acid; DHPC, 1,2-dihexadecyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; GP_{em}, emission generalized polarization; GP_{ex}, excitation generalized polarization; LAURDAN, 6-lauroyl-2-(*N,N*-dimethylamino)naphthalene; PRODAN, 6-propionyl-2-(*N,N*-dimethylamino)naphthalene; Sphin, egg yolk sphingomyelin.

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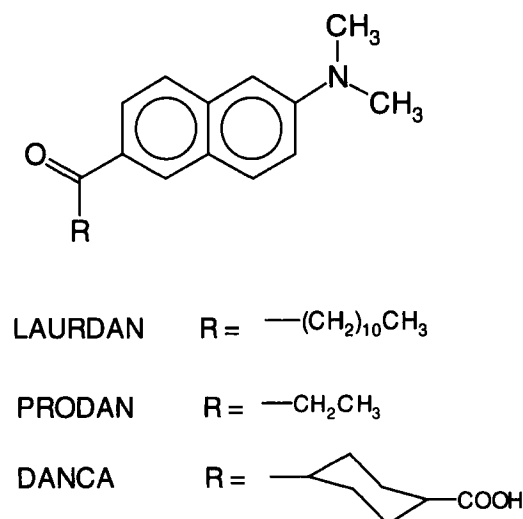


Figure 1. Chemical structures of LAURDAN, PRODAN and DANCA.

present at the lipid interface near LAURDAN's fluorescent moiety (4,5). Water molecules penetrate into the phospholipid interface during the main phase transition. To reorient with respect to LAURDAN's excited-state dipole, the rotational dynamics of these water molecules must occur in the same time scale as LAURDAN's fluorescence lifetime (3–4 ns). Part of LAURDAN's excited-state energy is utilized for the reorientation of the water dipoles causing an emission red shift (6). Clear evidence that water penetration is responsible for the dipolar relaxation observed in the liquid-crystalline phase of ester phospholipid vesicles is provided by the blue shift of LAURDAN's emission spectrum in D_2O with respect to the emission spectrum observed in H_2O at the same temperature (7). This blue shift is due to the slower rotational dynamics of D_2O compared to H_2O that results in less pronounced solvent dipolar relaxation in the phospholipid liquid-crystalline phase (7).

As in isotropic polar solvents, LAURDAN's excitation spectrum in ester phospholipid vesicles shows two well-separated electronic transition bands with maxima at 360 nm and 390 nm (4,5). In ester phospholipid vesicles in the gel phase, LAURDAN's red excitation band (390 nm) also corresponds to the excitation maximum (4). At temperatures above the main lipid phase transition the intensity of LAURDAN's red excitation band decreases and the excitation maximum is located in the blue excitation band (360 nm) (4). This temperature dependence of the intensity of LAURDAN's red excitation band in ester phospholipid vesicles has been attributed to differences in the packing of the lipid molecules (4,6). However, no attempts have been made to clarify the molecular basis of this phenomenon.

Due to the behavior of LAURDAN's fluorescence emission in ester phospholipid, *i.e.* the continuous red shift of the fluorescence emission during the lipid-phase transition, the excitation generalized polarization (GP_{ex}) function was defined analogous to the fluorescence polarization function (5) as

$$\text{GP}_{\text{ex}} = \frac{I_{\text{B}} - I_{\text{R}}}{I_{\text{B}} + I_{\text{R}}} \quad (1)$$

In this function, the relative parallel and perpendicular polarizer orientations in the classical polarization function were substituted by the intensities at the blue and red edges of the emission spectrum (I_{B} and I_{R} , respectively, in Eq. 1) using a given excitation wavelength (4–6). This parameter, GP_{ex} , contains information about solvent dipolar relaxation processes that occur during the time that LAURDAN is in the excited state and hence exhibits a large dipole moment and is related to water penetration in the phospholipid interfaces (see above) (4). Together with an extensive characterization of the behavior of the GP_{ex} function in ester phospholipid vesicles (4–7), the fluorescence emission characteristics of LAURDAN in ceramide-base lipid systems were recently reported (8,9). One of the most important conclusions of these latter studies was the inverse linear dependence of LAURDAN's GP_{ex} with the lipid intermolecular distance in pure aggregates of glycosphingolipid (neutral and charged) and phospholipids (8). The differences in water penetration in these systems are reflected by this general relationship and emphasize the strong sensitivity of LAURDAN's GP_{ex} function to water content and dynamics in lipid interfaces (7–10).

Because LAURDAN's excitation spectra in ester phospholipid systems vary depending on the lipid phase, *i.e.* gel or liquid crystalline, an emission generalized polarization (GP_{em}) function was defined (5) as

$$\text{GP}_{\text{em}} = \frac{I_{\text{R}} - I_{\text{B}}}{I_{\text{R}} + I_{\text{B}}} \quad (2)$$

where I_{R} and I_{B} are the intensities at the red and blue edges of the excitation spectrum using a given emission wavelength. The GP_{em} function gives information on the ground-state environment of the LAURDAN molecule.

LAURDAN's GP function was extensively used to characterize physicochemical events in pure lipid vesicles, mixtures of lipids and natural membranes (8–21). As discussed above, the molecular basis of LAURDAN's emission red shift in lipid vesicles is clearly related to the reorientation of water dipoles around the excited-state probe dipole (6–8). However, the molecular basis for the changes in LAURDAN's excitation spectrum in lipid interfaces, reflected in the LAURDAN GP_{em} function, are not fully understood. In this work we present a model that accounts for new, as well as previous, observations of the LAURDAN excitation spectrum's behavior in different kinds of lipid interfaces. This model provides a consistent picture of LAURDAN's ground-state characteristics when this probe is inserted into vesicles of various lipids.

MATERIALS AND METHODS

Materials. LAURDAN was from Molecular Probes, Inc. (Eugene, OR), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dihexadecyl-*sn*-glycero-3-phosphocholine (DHPC) were from Avanti Polar Lipids (Alabaster, AL) and sphingomyelin (Sphin) was from Sigma (St. Louis, MO).

Sample preparation. Stock solutions of LAURDAN, DPPC, DHPC and Sphin were made in chloroform. Lipid-probe dispersions were made with a lipid:probe ratio of 400:1 (LAURDAN final concentration was 1.5 μM). The solvent was evaporated under a stream of N_2 and the lipid was dried under high vacuum overnight. The dry lipid mixtures were hydrated in 20 mM Tris-HCl–50 mM NaCl, pH 7.4 above the main phase-transition temperature. All samples were

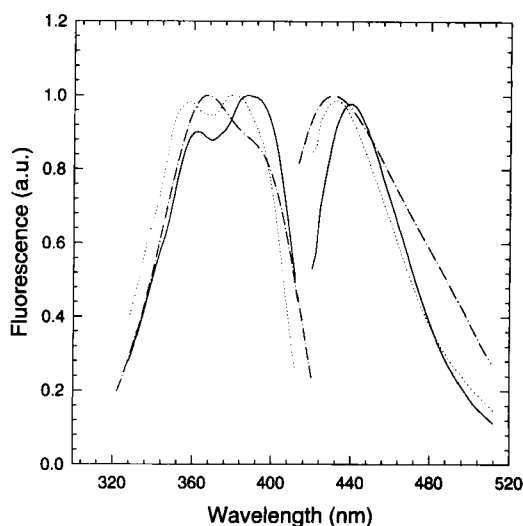


Figure 2. Normalized LAURDAN excitation and emission spectra below the lipid main-phase transition. DPPC (—), DHPC (.....) and Sphn (-----). The temperature was 23°C.

prepared and stored in the dark and spectra were measured immediately after preparation.

Fluorescence measurements. Steady-state excitation and emission spectra were measured with a PC1 photon counting spectrofluorometer (ISS Inc., Champaign, IL) equipped with a xenon-arc lamp using 2 nm bandwidth. The excitation spectra were corrected with a quantum counter (Rhodamine B in 3 g/mL ethylene glycol) in the reference cell, and the emission spectra were corrected for instrument response using the ISS software.

The GP_{ex} values were calculated by using 360 nm excitation wavelength and 440 nm and 490 nm wavelengths for the emission intensities (I_B and I_R , respectively, in Eq. 1). The GP_{em} values were calculated using 440 nm emission wavelength and 390 nm and 360 nm wavelengths for the excitation intensities (I_R and I_B , respectively, in Eq. 2). The lipid-phase-transition temperature was calculated from LAURDAN GP data using dGP/dT . The temperature was controlled by a thermostated bath to $\pm 0.1^\circ\text{C}$ and was directly measured in the sample cuvette by a digital thermometer.

LAURDAN's fluorescence lifetimes were measured by a cross-correlation, multifrequency, phase and modulation fluorometer (ISS Inc., Champaign, IL) using the harmonic content approach (22). Samples were excited with 345 nm light from the output of a cavity-dumped Rhodamine 6G dye laser (Coherent, Palo Alto, CA) synchronously pumped by a mode-locked Nd:YAG laser. The emission at wavelengths greater than 380 nm was observed through a KV399 cutoff filter. Excitation and emission path polarizers were set at 0° and 55° , respectively, to eliminate polarization artifacts in the fluorescence decay measurements (23). A solution of 1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene (dimethyl-POPOP) in ethanol was used as a lifetime reference, $\tau = 1.45$ ns. Data were collected until the standard deviation from each measurement of phase and modulation were at most 0.2° and 0.004, respectively. Phase and modulation data were analyzed using either discrete exponentials (24) or distribution models (25). In all cases the data fit best (as judged by the reduced chi-square) to two components: a major component, corresponding to a Lorentzian distribution and a minor discrete exponential component.

RESULTS

The fluorescence excitation and emission spectra of LAURDAN in DPPC, DHPC and Sphn vesicles in the gel and the liquid-crystalline phase are reported in Figs. 2 and 3, respectively. In the gel phase the fluorescence emission spectra of LAURDAN in DHPC and Sphn vesicles have maxima shifted to the blue with respect to DPPC (Fig. 2).

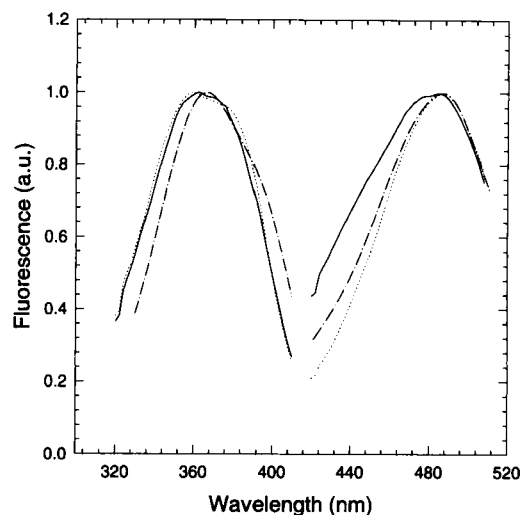


Figure 3. Normalized LAURDAN excitation and emission spectra above the lipid main-phase transition. DPPC (—), DHPC (.....) and Sphn (-----). The temperature was 50°C.

In addition, the LAURDAN's emission spectrum in Sphn and DHPC vesicles in the gel phase is broader than in DPPC. In the liquid-crystalline phase, LAURDAN's emission spectra in DHPC and Sphn are red-shifted compared to that found in DPPC vesicles (Fig. 3).

Below the gel \rightarrow liquid-crystalline phase transition LAURDAN's excitation spectra in DPPC, DHPC and Sphn vesicles present two bands (which we refer to as the blue and red bands) (Fig. 2). Relative to the blue band, the intensity of the red band decreases as DPPC > DHPC > Sphn. In DPPC and DHPC vesicles the excitation maximum corresponds to the red band while in Sphn the maximum is located in the blue band. In the liquid-crystalline state, LAURDAN's excitation spectrum in DHPC is similar to that found in DPPC vesicles at the same temperatures (Fig. 3). However, in Sphn vesicles LAURDAN's excitation spectrum is slightly red-shifted compared to those found in DPPC and DHPC vesicles (Fig. 3).

Figure 4 shows the temperature dependence of LAURDAN's GP_{ex} and GP_{em} in DHPC, DPPC and Sphn vesicles. The transition temperatures detected by LAURDAN GP_{ex} were 43°C, 41°C and 38°C for DHPC, DPPC and Sphn, respectively, in agreement with the data previously reported (Fig. 4a) (26,27). With respect to GP_{ex} , the GP_{em} function shows a lower sensitivity to the main phase-transition temperature (Fig. 4b). In DHPC vesicles the main phase-transition temperature is barely detectable. In Sphn the GP_{em} variation follows a temperature-dependent trend, with no indication of a lipid-phase transition.

The fluorescence lifetimes values of LAURDAN inserted in DHPC, DPPC and Sphn vesicles were measured below and above the main phase-transition temperature and the results are reported in Table 1. In all samples, the lifetime values were greater in the gel than in the liquid-crystalline phase in agreement with previous results (6–8). This finding shows that specific interactions with the lipid environment due to the different lipid structure in the two phases do not affect the LAURDAN excited-state lifetimes.

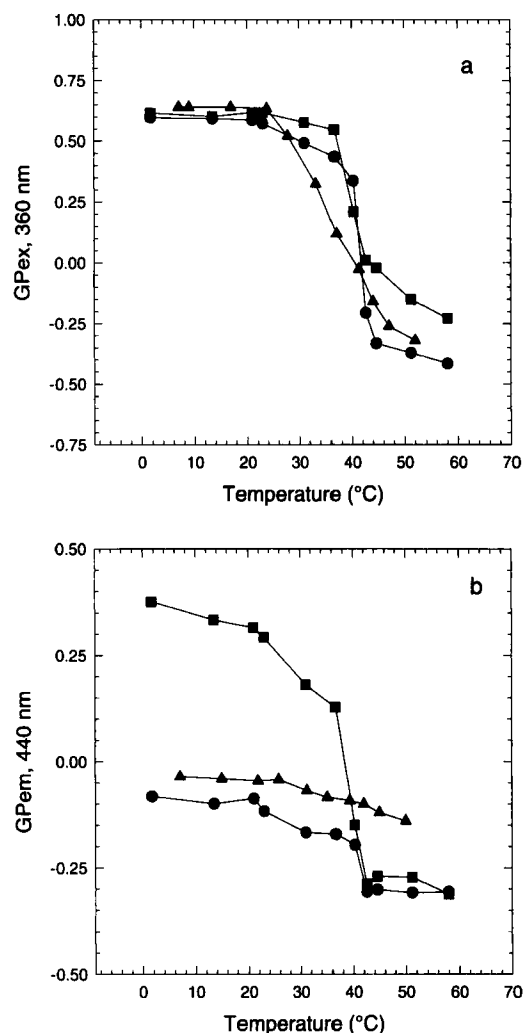


Figure 4. LAURDAN GP_{ex} (a) and GP_{em} (b) as a function of temperature in DPPC (■), DHPC (●) and Sphing (▲) vesicles.

DISCUSSION

In phospholipid membranes, the occurrence and the extent of dipolar relaxation depends upon the bilayer phase-state, *i.e.* upon lipid packing. Loose lipid packing, as in the liquid-crystalline phase, allows water molecules to penetrate into the lipid bilayer. The reorientation of water molecule dipoles around the excited-state dipole of LAURDAN is at the origin of the red shift during the excited-state lifetime of the probe's emission. In our experiments, differences in the emission spectra positions, and therefore in the GP_{ex} values, were found between DPPC and the other two lipids studied (DHPC and Sphing), suggesting more extensive dipolar relaxations for DHPC and Sphing than for DPPC in the liquid-crystalline state. The greater extent of water penetration sensed by LAURDAN in ether phospholipids, compared with that found in ester phospholipids above the main phase-transition temperature, is in keeping with the observations made among plasmalogens, ether phospholipid and ester phospholipid interfaces using PRODAN (28,29).

As shown in Fig. 2, the relative intensities of the red and the blue bands in LAURDAN's excitation spectra, vary depending on the lipid system. The ratio between the intensi-

Table 1. LAURDAN fluorescence lifetimes taken in the gel and the liquid crystalline phase of DPPC, DHPC and Sphing using excitation wavelength of 345 nm

Lipid	τ^* (ns) gel phase	τ^* (ns) liquid crystalline phase
DPPC	6.08	3.44
DHPC	5.91	3.33
Sphing	6.82	3.09

*Center of value of the Lorentzian distribution.

ties of the red and the blue band in the gel state decreases as follows: DPPC > DHPC > Sphing. The chemical bond between the fatty acid and the glycerol backbone in DPPC and DHPC (ester and ether, respectively) is the only difference between these two phospholipid molecules. Both, DPPC and DHPC form vesicles and have a very similar transition temperature (26). In the case of Sphing the polar head group is the same as in DPPC and DHPC, but the hydrophobic part is a ceramide moiety. Sphing, like DPPC and DHPC, forms vesicles when dispersed in water (8,27).

The LAURDAN excitation spectra in different solvents were reported by Parasassi *et al.* (4). These data suggest that the dielectric constant of the solvent does not correlate with the behavior of the LAURDAN red excitation band. For example, the red band of the LAURDAN excitation spectrum is intense in cyclohexanol ($\epsilon = 15.0$), ethanol ($\epsilon = 24.3$) and methanol ($\epsilon = 32.6$) (4). In acetone ($\epsilon = 20.7$), the intensity of the red band of the LAURDAN excitation spectrum is lower than that reported in alcoholic solvents. Moreover, the intensity of the LAURDAN red excitation band in nonpolar solvents is negligible. From these data it appears that other mechanisms are responsible for the appearance of this spectral feature. We suggest that the possibility to establish specific interactions (such as hydrogen bonds for example) between LAURDAN and the solvent molecules might be a more reasonable explanation for the increase of the intensity of the red excitation band in the LAURDAN excitation spectra. In phospholipid vesicles LAURDAN molecules are located parallel to the phospholipid chains with the fluorescence moiety located near the lipid glycerol backbone region (see Fig. 5) (6–8). Taking into account our spectroscopic observations, the above considerations about the LAURDAN behavior in solvents and the LAURDAN location in the membrane, we propose a model to explain our results. In this model the interactions between the dimethylamino residue of LAURDAN and specific functional groups of the lipids stabilize, to a greater or lesser extent, the electronic transition corresponding to the red excitation band. We believe that the high anisotropic environment presented by the gel-phase lipid membranes influences differently the interaction between LAURDAN and the functional groups of the lipid molecules compared with that found in isotropic solvents (4).

In the case of ester phospholipids, our model predicts a strong interaction between the dimethylamino moiety of LAURDAN and the ester linkages of the phospholipid molecules (Fig. 5a). Instead, the interaction between the dimethylamino residue of LAURDAN and the ether linkage

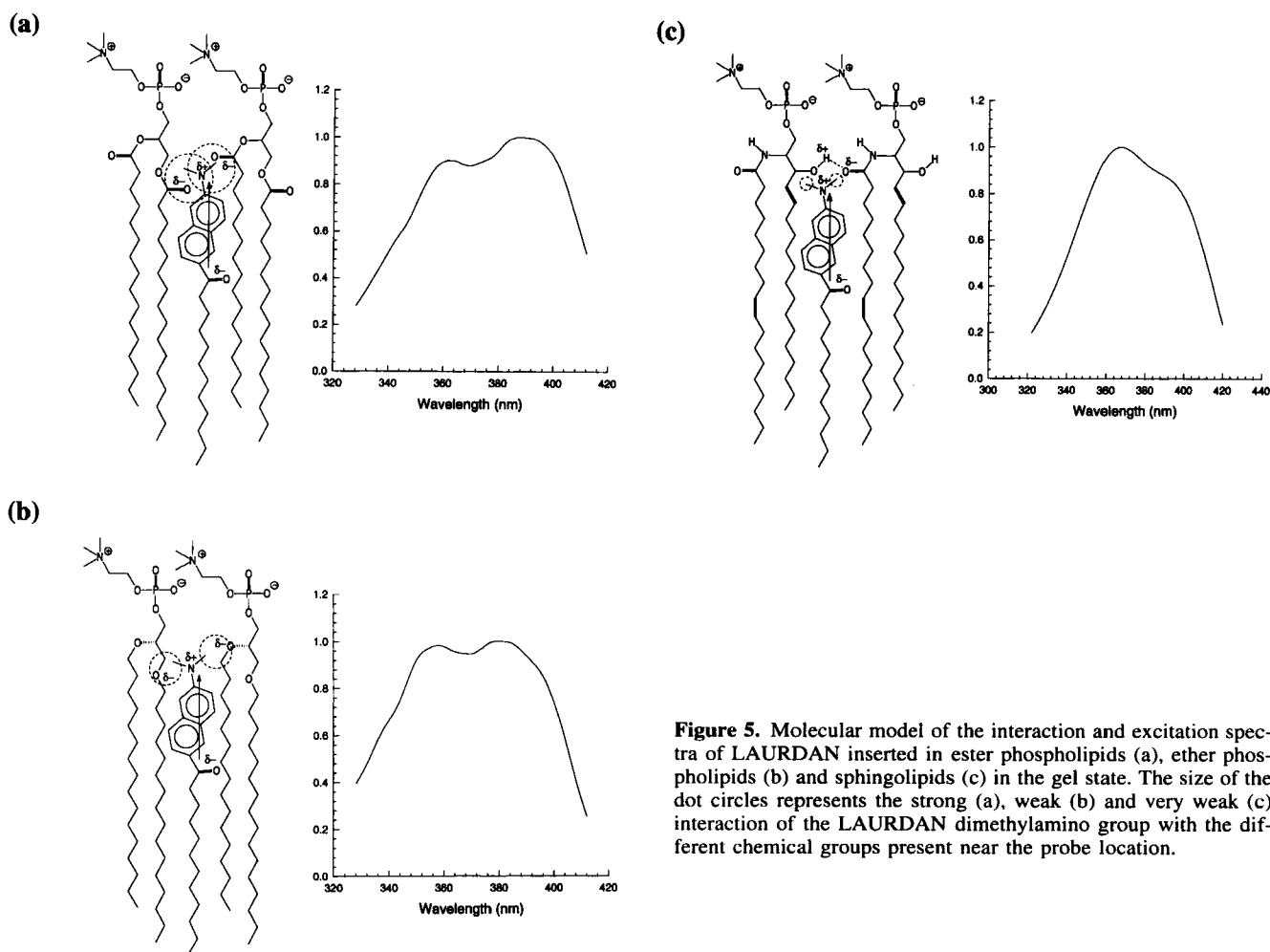


Figure 5. Molecular model of the interaction and excitation spectra of LAURDAN inserted in ester phospholipids (a), ether phospholipids (b) and sphingolipids (c) in the gel state. The size of the dot circles represents the strong (a), weak (b) and very weak (c) interaction of the LAURDAN dimethylamino group with the different chemical groups present near the probe location.

in ether phospholipid vesicles in the gel phase is, instead, weaker (Fig. 5b). In Sphin vesicles, LAURDAN can interact with the hydroxyl residue and the amide linkage present in the hydrophobic-hydrophilic interface (Fig. 5c). In this last case the strength of this interaction, as judged by relative intensities of the red and the blue excitation bands, seems to be very weak compared with that found in ester phospholipids (DPPC). The capability of the ceramide-base lipids to form interlipid hydrogen bonds (26) may reduce their ability to interact with LAURDAN. Similar characteristics of LAURDAN's excitation spectra were found in vesicles formed by simple polar head group ceramide-base lipids, *i.e.* cerebroside and sulfatide (8). However, it is interesting that in ceramide-base lipids with complex polar head group, *i.e.* gangliosides, LAURDAN's excitation spectrum in the gel phase shows a red band as a maximum with a slight decrease in the intensity of this band in the liquid-crystalline state. The intensity of LAURDAN's red excitation band in gangliosides is less than that found in DPPC (8,9). From the point of view of our model there should be a stabilization of LAURDAN's excitation red band in the ganglioside micelles caused by a particular interaction between the functional groups of the gangliosides at the top part of the ceramide moiety and the dimethylamino moiety of LAURDAN. Gangliosides form micelles and the micelle structure pos-

sesses high water content and a different lipid arrangement compared with vesicles (8,9). In ganglioside micelles, the formation of the interlipid hydrogen bonds is not found (26). This situation should permit LAURDAN to interact with the functional groups present in the top part of the ceramide moiety. These observations clearly point out that the physical characteristics of the lipid aggregates (shape, topology) together with the chemical structure of each particular lipid are the principal causes that affect the features of the LAURDAN excitation spectrum in lipid interfaces, in particular the behavior of the red excitation band.

Our model is also supported by studies of the effect of cholesterol in ester phospholipid interfaces on LAURDAN's spectral properties. The intensity of LAURDAN's red excitation band in ester phospholipids below the main phase-transition temperature decreases when cholesterol is added (30). Instead, cholesterol increases the intensity of LAURDAN's red excitation band in the lipid liquid-crystalline phase (30). Cholesterol is known to increase the packing between the ester phospholipid in the liquid-crystalline phase. The opposite effect occurs at temperatures below the main phase transition. Following our model we hypothesize that the effect of cholesterol on the packing of ester phospholipid vesicles in the different lipid phases (gel and liquid crystalline) modifies the capability of LAURDAN to interact

with the ester linkage, changing the intensity of the LAURDAN red excitation band.

Other evidence supporting our model comes from PRODAN's fluorescence behavior in ester phospholipid vesicles in the gel phase (31). PRODAN's excitation spectrum in ester phospholipid vesicles in the gel phase shows a maximum located in the blue excitation band with a low intensity red band (31). PRODAN has a propionic acid residue instead of LAURDAN's lauric acid residue (Fig. 1). In comparison to LAURDAN, the location of PRODAN in phospholipid bilayers is closer to the bulk water (32) and therefore, the possibility of an interaction between the dimethylamino group of PRODAN and the ester linkage of ester phospholipid is reduced.

Different sensitivity of LAURDAN's GP_{em} and GP_{ex} to the phase-transition temperature in different lipid interfaces

The interaction between LAURDAN and the glycerol backbone region in ester phospholipids is dependent on lipid packing. For example, the intensity of LAURDAN's red excitation band decreases during the phase transition in ester phospholipids (compare Fig. 2 with Fig. 3). According to our model, the increase in the mobility of the lipid molecules, together with the water penetration in the lipid vesicles in the liquid-crystalline phase, diminishes the strength of the interaction between the dimethylamino moiety of LAURDAN and the glycerol backbone region in ester phospholipids. Water can form hydrogen bonds with the ester groups of the lipids and hence compete with the electrostatic interaction between the ester groups and LAURDAN's dimethylamino moiety. This effect, though still present in ether phospholipids and Sphn, is diminished compared to that found in ester phospholipids. The relative insensitivity of the GP_{em} parameter to the phase transitions in these systems, compared to the ester phospholipid system, is due to the decreased strength of the probe-lipid interactions (see above and Fig. 4b). This latter fact influences the differences between the LAURDAN excitation spectra in the gel and the liquid-crystalline state and in consequence impede the GP_{em} function to detect the main phase transition. The LAURDAN's GP_{ex} function, on the other hand, is sensitive to excited-state dynamics and is opposed to the GP_{em} function that detects ground-state conformations. The GP_{ex} is able to detect clearly the lipid main-phase transition in systems that presents substantial changes in water content when the phase transition takes place, *i.e.* from the gel \rightarrow liquid crystalline (see above and Fig. 4a). When the changes in water content are not significant, such as in the case of gangliosides, the lipid main-phase transition is not detected by the LAURDAN GP_{ex} function (8,9).

Lack of sensitivity of LAURDAN's GP spectra to domain coexistence in Sphn

Recently, we reported the lack of sensitivity of LAURDAN's GP spectra (emission and excitation GP as a function of wavelength) to domain coexistence in Sphn and in glycosphingolipid series (8). The GP wavelength dependence at the phase-transition temperature in these ceramide-based lipid systems was opposite to that found in ester phos-

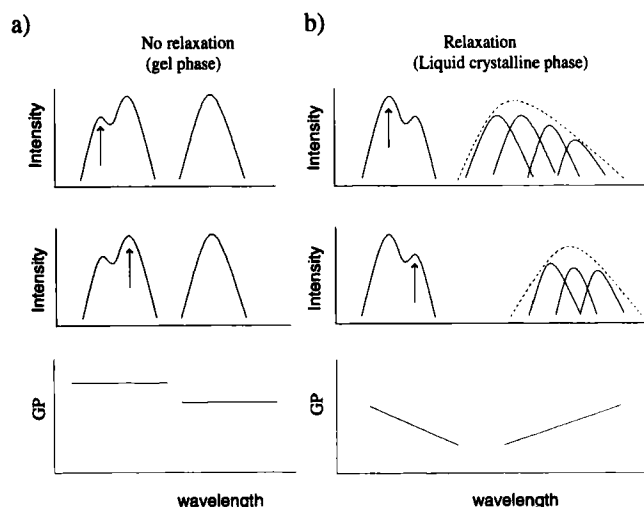


Figure 6. Sketch of LAURDAN's excitation and emission intensity and GP spectral behavior in ester phospholipid vesicles. Gel phase (a); liquid-crystalline phase (b).

pholipids (8). Our hypothesis about this observation, at that time, was based on the possibility of different types of interactions between the fluorescent probe and the lipids in these aggregates (8). We shall now reevaluate this hypothesis using the model presented above.

For clarification we shall first discuss the behavior of LAURDAN GP_{ex} and GP_{em} spectra in ester phospholipid vesicles. In the gel state, LAURDAN's emission spectrum is largely independent of the excitation wavelength due to the lack of dipolar relaxation processes (Fig. 6a) (4). This fact explains the wavelength independence of LAURDAN's GP_{em} and GP_{ex} values in ester phospholipid vesicles in the gel state (Fig. 6a) (4,6). This phenomenon is also found in ether phospholipids (not shown), Sphn and simple polar head group glycosphingolipids, such as cerebroside and sulfatides (8). However, in the gel phase of complex polar head group glycosphingolipids (gangliosides) the wavelength independence of LAURDAN's GP is not found. The dynamics and content of water in ganglioside micelles is comparable with those found in the liquid-crystalline state of phospholipid vesicles (8). Therefore, the dependence of the LAURDAN emission and excitation GP spectra in ganglioside micelles in the gel phase are similar to those found in phospholipid vesicles in the liquid-crystalline phase (see below) (8,9).

In the liquid-crystalline state, LAURDAN's emission spectrum depends on the excitation wavelength, because excitation in the blue part of the absorption spectrum preferentially photoselects molecules with partially relaxed surroundings, whereas excitation in the red part of the absorption spectrum preferentially photoselects molecules with surroundings already relaxed (Fig. 6b) (33). Therefore, the GP_{em} and GP_{ex} values are wavelength dependent in the liquid-crystalline phase, specifically, GP_{em} increases with increasing emission wavelengths (positive slope) and GP_{ex} decreases with increasing excitation wavelength (negative slope) (see Fig. 6b) (6). At the phase-transition temperature, however, the coexistence of gel and liquid-crystalline domains leads to a different wavelength dependence for GP_{em} and GP_{ex} ,

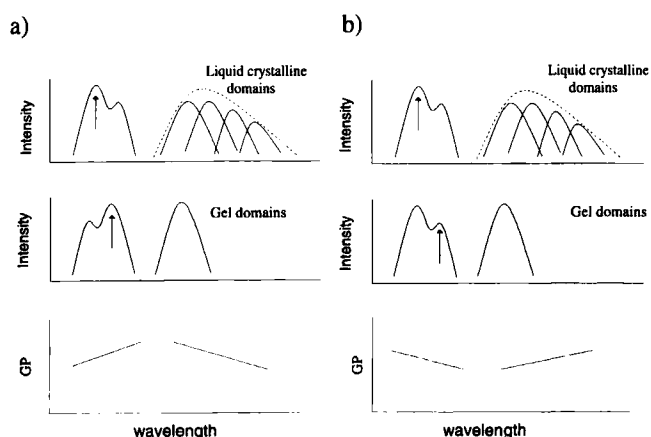


Figure 7. Sketch of LAURDAN's excitation and emission intensity and GP spectral behavior in ester phospholipid vesicles (a) and Sphing vesicles (b) at the lipid-phase transition temperature. At this temperature there are coexisting gel and liquid-crystalline phases in the lipid vesicles.

specifically, GP_{em} decreases with increasing emission wavelengths (negative slope) and GP_{ex} increases with increasing excitation wavelength (positive slope) (see Fig. 7a) (6). We note that the wavelength dependence of GP_{em} and GP_{ex} are opposite (6). This difference is due to the fact that the GP_{em} is calculated from the intensities at one particular emission wavelength exciting in the red and blue sides of the LAURDAN excitation spectra, while the GP_{ex} is calculated from the intensities in the blue and the red sides of the LAURDAN emission spectra obtained at one particular excitation wavelength (see Eqs. 1 and 2 and Figs. 6b and 7a).

Compared with ester phospholipids, a lack of sensitivity to phase coexistence is observed when LAURDAN is inserted into Sphing and simple polar head group glycosphingolipid vesicles (8). In these systems the wavelength dependence of LAURDAN GP_{em} and GP_{ex} at the transition temperature are similar to that found in ester phospholipids in the liquid-crystalline state (Fig. 7b) (8). In Sphing and simple polar head group glycosphingolipid vesicles we were not able to observe clear differences in the shape of LAURDAN's excitation spectra between the gel and the liquid-crystalline phase. In particular, the relative intensity change of the LAURDAN red excitation band between the gel and the liquid-crystalline phase in these systems is low compared with that found in ester phospholipid vesicles (see Fig. 2 and compare with Fig. 3) (8). The preferential photoselection (high intensity red excitation band) that operates in the case of ester phospholipids and to a lesser extent in ether phospholipids in the gel state is due to the favorable interactions between the fluorescence probe and the lipid molecules in the ground state (see above). This preferential photoselection of LAURDAN molecules in the more rigid domains is a necessary condition to obtain the positive slope in the LAURDAN GP_{ex} spectrum and the negative slope in the LAURDAN GP_{em} spectrum at the phase-transition temperature.

CONCLUSIONS

We propose a model to explain the behavior of LAURDAN red excitation band in ester and ether phospholipids and

Sphing vesicles at different temperatures (note that all these lipids have the same polar head group). The model takes into account interactions between the dimethylamino moiety of LAURDAN and the functional groups of the lipid molecules near the probe location in the lipid aggregates. This interaction of lipids with LAURDAN stabilizes, in different ways, LAURDAN's ground state and explains the different behavior of the GP_{em} function depending on the nature of the lipid molecules. Our model fits well with previous observations on the behavior of LAURDAN's excitation spectrum in glycosphingolipids and cholesterol-containing phospholipid interfaces. Also the characteristics of PRODAN's fluorescence excitation spectra in ester phospholipid vesicles fits with our model. With this model we can comprehend precisely the information contained and the behavior of the GP_{em} function in various lipid systems. We want to point out that LAURDAN's sensitivity to the lipid chemical environment of lipid systems is observed using ground-state information.

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