Partitioning of Amphiphiles between Coexisting Ordered and Disordered Phases in Two-Phase Lipid Bilayer Membranes

Rute M. R. S. Mesquita,* Eurico Melo,* Thomas E. Thompson,† and Winchil L. C. Vaz‡
*Instituto de Tecnologia Química e Biológica, P-2780 Oeiras, Portugal; †University of Virginia, Charlottesville, Virginia 22908 USA; and ‡Departamento de Química, Universidade de Coimbra, P-3000 Coimbra, Portugal

ABSTRACT The partition coefficients (K_P) of a series of single-chain and double-chain fluorescent amphiphiles, between solid ordered ($P_{\beta'}$ and L_β) and liquid disordered (L_α of the type l_d) lipid phases coexisting in the same lipid bilayer, was studied using steady-state fluorescence emission anisotropy. The single-chain amphiphiles were *N*-(7-nitrobenzoxa-2, 3-diazol-4-yl)-alkylamines, and the double-chain amphiphiles were *N*-(7-nitrobenzoxa-2, 3-diazol-4-yl)-phosphatidylethanolamines with chain lengths of 12–18 carbon atoms. Saturated 18-carbon alkyl/acyl chain compounds were also compared with Δ^9-*cis* unsaturated chains of the same chain length. The fluorescence anisotropy of the probes was examined in lipid bilayers (multilamellar vesicles) prepared from an equimolar mixture of dilauroylphosphatidylcholine and distearoylphosphatidylcholine and studied as a function of temperature through the entire temperature range of coexistence of ordered gel phases and a disordered fluid phase in this system. The unsaturated chain amphiphiles partitioned exclusively into the fluid phase whenever this phase was present, as did the saturated chain amphiphiles with the shortest chains ($C_{12:0}$), while K_P ranges between 1 and 2, in favor of the L_β solid phase, for the amphiphiles with long saturated ($C_{18:0}$) alkyl/acyl chains, with intermediate behavior for the intermediate chain lengths. All probes appeared to be totally excluded from $P_{\beta'}$ solid (gel) phases. The technique was also used to determine partitioning of some of the probes between coexisting liquid ordered (cholesterol-containing) (l_o) and liquid disordered (l_d) L_α phases. In this case the ratio of signal amplitude to noise allowed us to obtain a qualitative, but not quantitative, measure of the phase partitioning of the probes. We conclude that the partitioning behavior of the probes examined between coexisting l_o and l_d phases is qualitatively similar to that observed between solid ordered and liquid disordered phases.

INTRODUCTION

The possibility that the biological membrane is a heterogeneous chemical system (Vaz and Almeida, 1993) raises some interesting questions about the way in which its components are distributed in this structure and the physiological consequences of a heterogeneous distribution for the cell. Evidence has accumulated in the literature (Brown and Rose, 1992; Lisanti et al., 1994; Simons and Ikonen, 1997; Varma and Mayor, 1998) in support of biological membrane heterogeneity, with possible functional consequences, although this view is not uncontested (Kurzchalia et al., 1995; Kenworthy and Edidin, 1998). Based on simple physical-chemical principles, we have discussed the possible relevance of membrane heterogeneity for processes that occur in membranes and suggested some possible physiological consequences (Vaz, 1992, 1994, 1995, 1996; Melo et al., 1992; Thompson et al., 1995). However, the fact remains that the issue of whether biological membranes are heterogeneous chemical systems or not and whether the putative heterogeneity has any physiological consequences for the cell still begs a clear proof pro or contra.

With the development and application of high-resolution techniques such as near-field scanning optical microscopy (Hwang et al., 1998; Hollars and Dunn, 1998) and the analysis of results obtained from single-particle-tracking techniques (Saxton and Jacobson, 1997), the proof of the existence (or nonexistence) of biological membrane heterogeneity may be just around the corner. These techniques use membrane-bound amphipathic probes that are usually anchored to the membrane through insertion of some aliphatic chain that is part of the probe structure. It therefore becomes important to understand how amphiphilic probes are distributed in a membrane if it is heterogeneous. This question has been addressed in the past for several different kinds of amphiphiles by various authors (Sklar et al., 1979; Klausner and Wolf, 1980; Welti and Silbert, 1982; Huang et al., 1988; Tocanne, 1989; Spink et al., 1990; Martin et al., 1990; Dibble et al., 1993; Welti and Glaser, 1994; Feigenson, 1997). However, we are not aware of any study that systematically relates the structural characteristics (aliphatic chain length and degree of saturation) of a homologous series of probes to their partition coefficients between coexisting membrane phases beyond an "order-of-magnitude" estimate. A study by Huang et al. (1988) has determined partition coefficients (between liquid disordered and solid

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Address reprint requests to Prof. Winchil L. C. Vaz, Departamento de Química, Universidade de Coimbra, P-3000 Coimbra Codex, Portugal. Tel.: +351-239-824861; Fax: +351-239-827703; E-mail: wvaz@ci.uc.pt.

Abbreviations used: K_P, Solid/fluid phase partition coefficient; di-$C_{12:0}$PC, dilauroylphosphatidylcholine; di-$C_{14:0}$PC, dimyristoylphosphatidylcholine; di-$C_{18:0}$PC, distearoylphosphatidylcholine; NBD-, *N*-(7-nitrobenzoxa-2,3-diazol-4-yl)-; NBD-di$C_{n:x}$PE, NBD-labeled phosphatidylethanolamines, where *n* is the number of carbon atoms in the acyl chains and *x* is the number of double bonds; NBD-$C_{n:x}$, NBD-labeled alkylamines, where *n* is the number of carbon atoms in the alkyl chain and *x* is the number of double bonds.

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phases) for a homologous series of anthroyl-alkanoates in which the anthroyl reporter group is placed at various depths in the lipid bilayer. In the present work we relate the partition coefficients of homologous series of monoalkyl and diacyl amphiphiles, in a membrane with coexisting solid and fluid phases, to probe chain length and saturation as well as to determine the number of alkyl/acyl chains in the probe structure.

Membrane heterogeneity, of course, may be of various types. Several lipid lamellar phases that may exist in biological membranes have been clearly identified and characterized. Lipid bilayers can exist as highly ordered solid (gel) phases, highly disordered fluid phases, or relatively ordered (usually cholesterol-rich) fluid phases, and it is clear that under different conditions a lipid bilayer membrane can exist as any possible combination of the above phases. Possibly the most relevant phase coexistence from the biological perspective is that of liquid ordered and liquid disordered phases that coexist in the same membrane, and the least relevant one is that of solid/solid coexistence. In this work we report quantitative results on the phase partitioning of amphiphiles between the coexisting phases in membranes with solid/liquid disordered phase coexistence and some qualitative results on liquid ordered/liquid disordered phase coexistence.

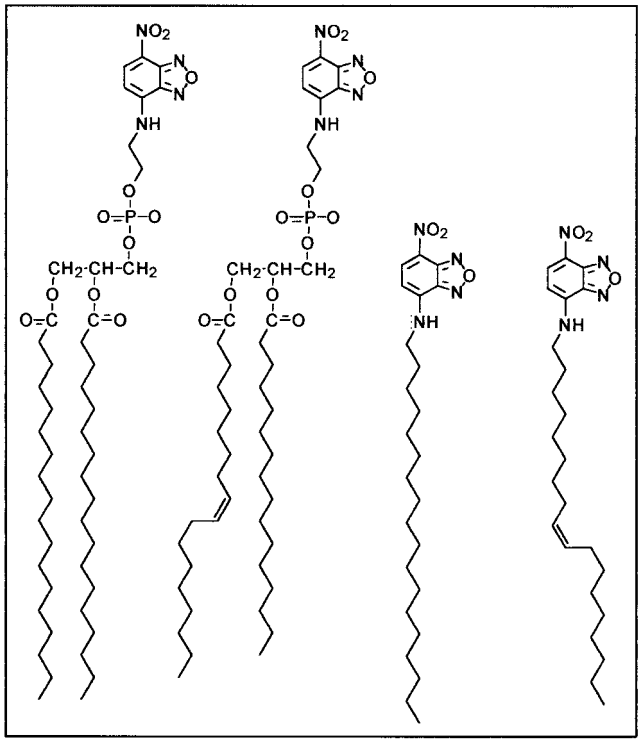

FIGURE 1 Representative structures of the probes used.

MATERIALS AND METHODS

The phosphatidylcholines and NBD-PEs (with the exception of NBD-di-$C_{12:0}$PE and NBD-di-$C_{18:0}$PE) used in this work were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further treatment. NBD-di-$C_{12:0}$PE and NBD-di-$C_{18:0}$PE were prepared as described by Vaz and Hallmann (1983). NBD-$C_{16:0}$ was purchased from Molecular Probes (Eugene, OR). All other NBD-alkylamines were prepared by reacting the respective alkylamines and NBD-chloride (both products of Sigma Chemical Company, St. Louis, MO) as described by Vaz and Hallmann (1983). Structures of representative probes used in this work are shown in Fig. 1.

Aqueous phospholipid suspensions were prepared by premixing the fluorescent amphiphiles and phosphatidylcholines in the desired molar proportions (1:100 or 1:300) as solutions in chloroform and evaporating the solvent in a stream of nitrogen at 70°C, followed by placing the residue in a vacuum dessicator (~15 Torr) for at least 2 h at room temperature. The residual lipid film, warmed to 70°C in a water bath, was hydrated with a 0.05 M aqueous solution of potassium chloride containing 0.02% sodium azide that had been preheated to the same temperature. The multilamellar vesicle suspension was vortexed for 2 × 15 s and left at 70°C for a period of 2 h before use in fluorescence measurements.

Fluorescence emission and excitation spectra were obtained on a thermostatted Spex Fluorolog F212E spectrofluorimeter, using 2 nm slits. Stirred multilamellar vesicle suspensions were used in these measurements. Quantum yields were obtained by integration of emission spectra that were corrected for instrumental distortions. The values of quantum yields are reported (see Table 1) relative to the quantum yield of NBD-$C_{16:0}C_{18:1\text{-(cis)}}$PE in di $C_{12:0}$PC membranes at 20°C, which was the system with the highest quantum yield.

Fluorescence anisotropy measurements were performed in a polarization fluorimeter specially constructed by us for these experiments. The fluorimeter has a T-geometry, computer-controlled temperature scanning between 10° and 70°C, and continuous sample agitation. Temperature scans were routinely performed at a rate of 0.25°C/min, but scan rates as low as 0.06°C/min were also occasionally used with no observable difference in the results. Polarization in the excitation and emission paths was accomplished with Glan-Taylor polarizers purchased from Melles Griot (Zevenaar, the Netherlands). Excitation was at 465 nm, with an Aminco single-grating monochromator with a 1.5-mm slit combined with a cutoff filter (KV418) from Schott and Gen (Mainz, Germany). Emission was measured at 530 nm with two bandpass filters (P10–530 nm from Corion, Franklin, MA) with a maximum transmittance of 54% and a bandwidth at half-maximum transmittance of 9 nm. Anisotropy was calculated using the expression

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2\,GI_{VH}} \tag{1}$$

where I_{VV} and I_{VH} are the parallel and perpendicular polarized fluorescence intensities measured with the excitation polarizer in a given orientation and I_{HH} and I_{HV} are the same fluorescence intensities measured with the excitation polarizer in an orientation at 90° with respect to the previous measurement. G is a correction factor given by $G = I_{HV}/I_{HH}$.

Partition coefficients, K_p, were calculated using the expression

$$K_p = \frac{x_s}{x_f}\frac{A_f}{A_s} \tag{2}$$

where x are the molar fractions of the probe and A are the fractional areas of the solid (subscript s) and fluid (subscript f) phases, respectively; with $x_s + x_f = 1$. The fractional areas of the two phases are calculated from their respective mass fractions, obtained by application of the lever rule to the phase diagram for the di-$C_{12:0}$PC/di-$C_{18:0}$PC mixture (Mabrey and Sturtevant, 1976), and assuming that the area per molecule of the solid phase is the same as for pure di-$C_{18:0}$PC bilayers and that of the fluid phase is the same as for pure di-$C_{12:0}$PC bilayers. Molecular areas for pure di-$C_{12:0}$PC and di-$C_{18:0}$PC bilayers at 25°C were obtained from Marsh (1990). The

TABLE 1 Emission spectral properties of the NBD-labeled amphiphiles used in this work

Amphiphile	Properties	In DSPC bilayers			In DLPC bilayers		
		20°C	30°C	60°C	20°C	30°C	60°C
NBD-di$C_{18:0}$PE	Emission λ_{max} (nm)	542	543	543	542	544	544
	Rel. quantum yield*	0.88	0.85	0.54	0.95	0.83	0.45
NBD-di$C_{16:0}$PE	Emission λ_{max} (nm)	545	546	546	542	544	544
	Rel. quantum yield*	0.81	0.76	0.53	0.90	0.84	0.46
NBD-di$C_{14:0}$PE	Emission λ_{max} (nm)	544	545	544	542	543	542
	Rel. quantum yield*	0.71	0.71	0.58	0.97	0.87	0.48
NBD-di$C_{12:0}$PE	Emission λ_{max} (nm)	544	545	544	542	543	543
	Rel. quantum yield*	0.61	0.52	0.53	0.98	0.86	0.46
NBD-$C_{16:0}C_{18:1\text{-}(cis)}$PE	Emission λ_{max} (nm)	546	548	544	543	544	544
	Rel. quantum yield*	0.43	0.46	0.56	1.00	0.89	0.49
NBD-$C_{18:0}$	Emission λ_{max} (nm)	550	552	549	548	549	549
	Rel. quantum yield*	0.53	0.48	0.38	0.82	0.68	0.35
NBD-$C_{16:0}$	Emission λ_{max} (nm)	548	550	547	548	549	548
	Rel. quantum yield*	0.66	0.58	0.41	0.75	0.67	0.36
NBD-$C_{14:0}$	Emission λ_{max} (nm)	548	549	548	547	548	548
	Rel. quantum yield*	0.59	0.51	0.40	0.78	0.63	0.35
NBD-$C_{12:0}$	Emission λ_{max} (nm)	536	539	543	548	548	548
	Rel. quantum yield*	0.41	0.43	0.42	0.86	0.76	0.39
NBD-$C_{18:0\text{-}(cis)}$	Emission λ_{max} (nm)	548	547	548	547	548	548
	Rel. quantum yield*	0.32	0.31	0.38	0.79	0.67	0.35

*Error for relative quantum yields is $\leq \pm 0.05$.

molar fractions of the probes in solid and fluid phases are related to the measured values of anisotropy in the mixed-phase system by the expression (Lentz et al., 1976)

$$r_{mix} = r_s\left(\frac{x_s \Phi_s}{x_s \Phi_s + x_f \Phi_f}\right) + r_f\left(\frac{x_f \Phi_f}{x_s \Phi_s + x_f \Phi_f}\right) \qquad (3)$$

where r are the measured anisotropies and Φ are the relative emission intensities. The anisotropies for the pure fluid and pure solid phases were measured using the probes NBD-di-$C_{18:0}$PE (as a reference for the two-chain probes) and NBD-$C_{16:0}$ (as a reference for the single-chain probes) incorporated into bilayers prepared from pure di-$C_{12:0}$PC (for r_f) and in pure di-$C_{18:0}$PC (for r_s), respectively. Φ_s and Φ_f were evaluated for each probe/lipid system by integrating the overlap area of the transmission curve of the emission bandpass filter and the respective emission spectrum.

RESULTS AND DISCUSSION

We chose to use lipid bilayers prepared from an equimolar binary mixture of di-$C_{12:0}$PC and di-$C_{18:0}$PC to study the phase partitioning behavior of the amphiphilic fluorescent probes, listed in the previous section, between solid ordered (P_β' and L_β) and liquid disordered (L_α or l_d) phases. This particular system was chosen because its temperature-composition phase diagram has been well defined (Mabrey and Sturtevant, 1976), and solid ordered and liquid disordered phases coexist in these bilayers over a wide range of temperature and composition. For convenience, the published phase diagram is shown in Fig. 2. Furthermore, the coex-

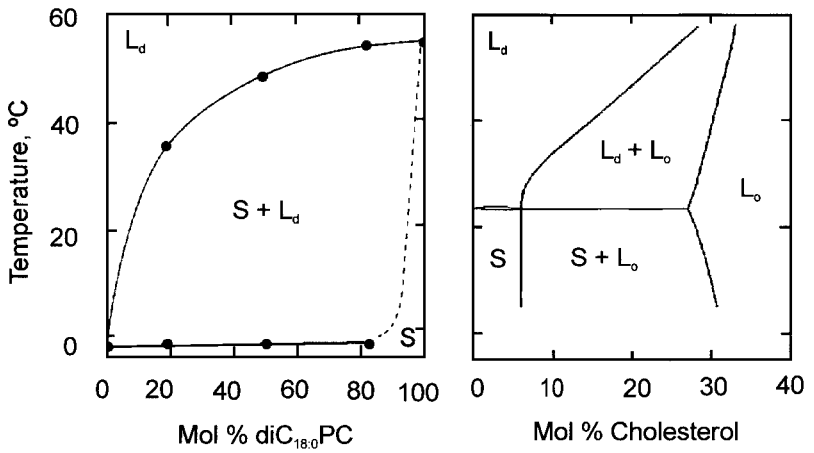

FIGURE 2 Temperature-composition phase diagrams for binary mixtures of (*A*) di-$C_{18:0}$PC and di-$C_{12:0}$PC (taken from Mabrey and Sturtevant, 1976) and (*B*) di-$C_{14:0}$PC and cholesterol (taken from Almeida et al., 1992).

isting solid and fluid phases, in the coexistence region of this phase diagram, are almost pure distearoylphosphatidylcholine and dilauroylphosphatidylcholine, respectively, which permits the use of bilayers prepared from these pure lipids as systems of reference. Studies that required coexisting liquid ordered (l_o) and liquid disordered (l_d) phases utilized bilayers prepared from binary mixtures of di-$C_{14:0}$PC and cholesterol for which a detailed temperature-composition phase diagram is also available (Almeida et al., 1992). In this case all measurements were made at 30°C, using bilayers prepared from pure di-$C_{14:0}$PC, a 65:35 molar ratio mixture of di-$C_{14:0}$PC and cholesterol, and a 82.5:17.5 molar ratio mixture of di-$C_{14:0}$PC and cholesterol, used, respectively, as models for membranes in the pure liquid disordered (l_d) phase, the pure liquid ordered (l_o) phase, and liquid ordered/liquid disordered phase coexistence.

Fig. 3 shows the emission spectra for NBD-di-$C_{18:0}$PE and NBD-$C_{18:0}$ amphiphiles inserted into di-$C_{18:0}$PC bilayers in the L_α and L_β phases. The emission maxima and relative emission quantum yields of all amphiphilic probes used in this work are summarized in Table 1. The spectra in Fig. 3 clearly show a significantly reduced relative emission quantum yield for the single-chain probes compared to the two-chain probes at the same temperature. This tendency is seen for almost all cases examined, listed in Table 1. In addition to the reduced quantum yields, there is a significant redshift (of between 4 and 10 nm) for the single-chain probes compared to the two-chain probes. This is seen for all of the probes except for those with very short chains ($C_{12:0}$) and with unsaturated chains ($C_{18:1(cis)}$ and $C_{16:0}C_{18:1(cis)}$). These observations suggest that the location of the probes is not identical in all cases; perhaps the probes with two chains are located in a slightly more aqueous region, and the probes with a single chain are in a location more in the region of the headgroup dipoles. Without significantly more information on the spectral properties of these systems, however, this conclusion must be considered a suggestive speculation.

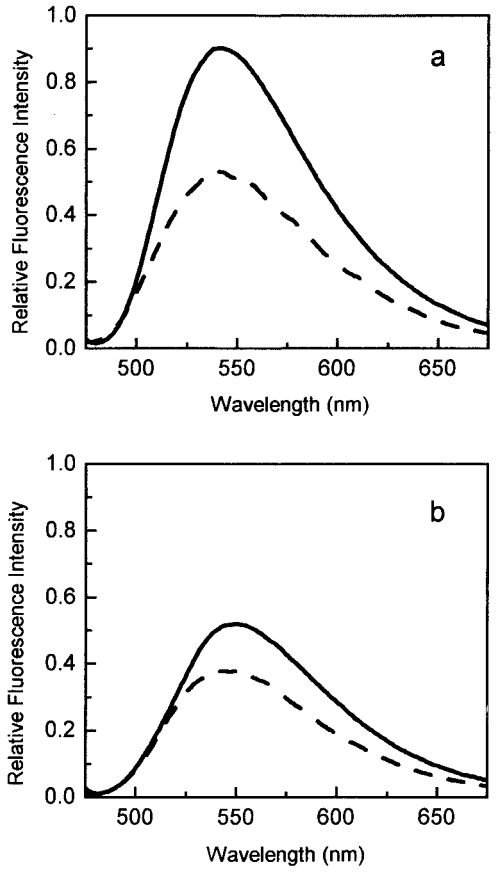

FIGURE 3 Fluorescence emission spectra of NBD-labeled amphiphiles in di-$C_{18:0}$PC bilayers. (*a*) NBD-di-$C_{18:0}$PE in di-$C_{18:0}$PC bilayers at 20° (——) and at 60°C (– – –). (*b*) NBD-$C_{18:0}$ in di-$C_{18:0}$PC bilayers at 20° (——) and at 60°C (– – –). Excitation was at 465 nm. These emission spectra are illustrated in separate panels for purposes of clarity. They may be directly superimposed for comparison. For further details concerning the spectral properties of all probes used in this work, the reader is referred to Table 1.

The fluorescence emission anisotropies of NBD-di$C_{18:0}$PE and NBD-$C_{16:0}$ in bilayer membranes prepared from pure di-$C_{12:0}$PC or di-$C_{18:0}$PC as a function of temperature are shown, for heating and cooling runs, in Fig. 4. For any given temperature the emission anisotropy values obtained in gel-phase di-$C_{18:0}$PC bilayers ($P_{\beta'}$ and L_β phases) and in liquid disordered phase di-$C_{12:0}$PC bilayers (L_α phase) represent the extremes of anisotropy values expected for the respective phases in the pure systems and in bilayers prepared from equimolar mixtures of di-$C_{12:0}$PC and di-$C_{18:0}$PC, because, as will be seen later, the probes with long saturated chains are soluble in both gel and fluid phases. In pure gel phases the anisotropy as a function of temperature showed differences between different probes, depending on their chain lengths and degree of saturation (data not shown). This was attributed to the fact that shorter and unsaturated chains tend to be excluded from pure gel-phase bilayers (see below). Probe emission anisotropies were also different for probes with one or two apolar chains (compare Fig. 4, *a* and *b*). It is also noteworthy that a distinct hysteresis (comparing heating and cooling curves) was observed for the temperature dependence of the emission anisotropy at a temperature corresponding to the $P_{\beta'} \rightarrow L_\beta$ transition. The hysteresis was more pronounced for the probe with two apolar chains than for the probe with a single apolar chain. This hysteresis may be related to the slow lipid packing rearrangements known to occur in this transition (Yao et al., 1991) and to the way in which the probes dissolve in the respective lipid solvent matrices.

In Fig. 4 we also show the fluorescence emission anisotropy of NBD-di$C_{18:0}$PE (Fig. 4 *a*) and NBD-$C_{16:0}$ (Fig. 4 *b*) in bilayers made from an equimolar mixture of di-$C_{12:0}$PC and di-$C_{18:0}$PC as a function of temperature. An interesting aspect of these results is that the anisotropy curves for

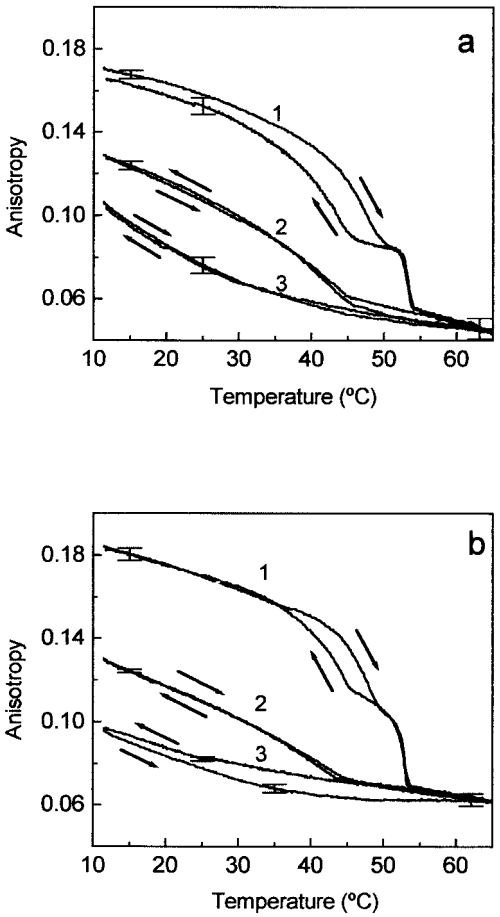

FIGURE 4 Fluorescence emission anisotropy as a function of temperature for (*a*) NBD-di-$C_{18:0}$PE in di-$C_{18:0}$PC bilayers (*1*), di-$C_{12:0}$PC bilayers (*3*), and bilayers prepared from an equimolar mixture of di-$C_{18:0}$PC and di-$C_{12:0}$PC (*2*); and for (*b*) NBD-$C_{16:0}$ in di-$C_{18:0}$PC bilayers (*1*), di-$C_{12:0}$PC bilayers (*3*), and bilayers prepared from an equimolar mixture of di-$C_{18:0}$PC and di-$C_{12:0}$PC (*2*). Experimental conditions are described in the Materials and Methods section. The arrows show the direction of the temperature scan.

mixed lipid bilayers in Fig. 4 only begins to show the effect of partitioning of the probes into the ordered phase when there is already ~20% ordered phase in the system (at ~45°C). Considering that the ordered phase that coexists with the L_α fluid phase in this system at this temperature is almost pure di-$C_{18:0}$PC, we propose that this effect may be the result of the ordered phase being a $P_{\beta'}$ gel phase, into which neither of the probes partitions, preferring solution in the coexisting L_α fluid phase. However, this absolute preference for dissolution in the L_β phase is not found for the case in which the L_α gel phase coexists with the L_β fluid phase (temperatures below ~45°C).

Table 2 lists the values of K_P obtained in this work for a homologous series of NBD-PEs, with acyl chains between $C_{12:0}$ and $C_{18:0}$, in lipid bilayers constituted from an equimolar mixture of di-$C_{12:0}$PC and di-$C_{18:0}$PC. The values are given at 20°C and 30°C, at which temperatures the system has coexisting solid ordered (L_β) and liquid disordered (L_α of the type l_d) phases in the same bilayer. The amphiphiles with the shortest acyl chains are totally excluded from the gel phase. K_P progressively increases with increasing acyl chain length; the compound with the longer acyl chains ($C_{16:0}$ and $C_{18:0}$) is essentially indifferent to the gel or fluid phase, or prefers the gel phase slightly. A Δ^9-*cis*- double bond in one of the acyl chains is sufficient to make the amphiphile prefer the fluid phase (compare NBD-di$C_{18:0}$PE with NBD-$C_{16:0}C_{18:1(cis)}$PE and NBD-$C_{18:0}$ with NBD-$C_{18:1(cis)}$ in Table 2). The preference of short-chain amphiphiles for fluid-phase bilayers and long-chain amphiphiles for gel-phase bilayers had been shown, albeit qualitatively, several years ago by Klausner and Wolf (1980). The result may be understood in terms of a destabilizing effect of very short or unsaturated acyl chains upon the lipid order in the gel phase and their consequent exclusion from this phase. As the acyl chain length and configuration approach those of the acyl chain of the host gel-phase bilayer, the packing incompatibility decreases with a consequent tendency of the amphiphiles to dissolve better in the gel phase. The inability of the fluorescent amphiphiles to partition into $P_{\beta'}$ gel phases probably has to do with the interactions in the polar headgroup region of the lipids forming the bilayer. The bulky fluorescent probe in the polar part of these molecules does not favor a nonperturbing presence of these amphiphiles in this gel phase. Single-chain amphiphiles show a similar tendency with regard to their preference for gel or fluid phases as the two-chain amphiphiles.

TABLE 2 Partition coefficients of NBD-labeled amphiphiles between ordered solid and disordered fluid phases

Amphiphile	Temp. °C	$K_{P(S/F)}$* Heat	Cool
NBD-di$C_{18:0}$PE	20	1.0	1.1
	30	1.3	1.4
NBD-di$C_{16:0}$PE	20	1.0	1.1
	30	1.1	1.2
NBD-di$C_{14:0}$PE	20	0.4	0.5
	30	0.5	0.5
NBD-di$C_{12:0}$PE	20	0.0	0.0
	30	0.0	0.0
NBD-$C_{16:0}C_{18:1-(cis)}$PE	20	0.0	0.0
	30	0.0	0.1
NBD-$C_{18:0}$	20	1.7	1.5
	30	2.5	1.7
NBD-$C_{16:0}$	20	1.0	0.9
	30	1.3	1.3
NBD-$C_{14:0}$	20	0.5	0.6
	30	0.6	0.7
NBD-$C_{12:0}$	20	0.2	0.5
	30	0.2	0.6
NBD-$C_{18:1-(cis)}$	20	0.1	0.5
	30	0.4	0.6

*Error for K_p is $\leq \pm 0.1$.

The results are also summarized in Table 2. There seems to be a slight tendency in this case for the single-chain amphiphiles to dissolve better in the gel phase.

It is worth noting that the values of K_P are generally higher at 30°C than they are at 20°C for all probes examined. This may be understood if we assume that the apparent partitioning of the amphiphiles into the solid-phase (L_β) bilayer domains is actually due to their inclusion in defect structures (grain boundaries and point defects). Such defects may be expected to be fewer at the lower temperature, with a consequently greater exclusion of the probes from these domains at 20°C. In this context it is also interesting to note that single-chain amphiphiles apparently partition more into the solid-phase (L_β) domains than the two-chain amphiphiles. This could also be explained by a more facile inclusion of single-chain amphiphiles into single point defects.

Table 3 shows some results on probe fluorescence emission polarization in liquid disordered (l_d), liquid ordered (l_o), and mixed liquid ordered/liquid disordered bilayers. The phase diagram for the system used (di-$C_{14:0}$PC/cholesterol) is shown in Fig. 2. The differences between the extreme values of anisotropy are quite low (0.076 for the l_d phase and 0.128 for the l_o phase). However, they do allow us to make a qualitative judgment of how the two probes examined (fully saturated and Δ^9-*cis*-unsaturated) partition in a membrane in which these two phases coexist. Qualitatively, the probe with the saturated chain appears to prefer the liquid ordered phase, while the probe with the unsaturated chain seems to prefer the liquid disordered phase. In this connection, we (Pokorny et al., 1999) have recently reported a study on the association of a similar amphiphile with a $C_{16:0}$ aliphatic chain with membranes having l_o-l_d phase coexistence. In this work a K_P of ~0.2 was estimated for the probe partitioning between l_o and l_d phases, favoring the l_d phase.

Finally, we may attempt a speculation at this point as to the effects of attaching a single acyl chain or two acyl chains to a globular particle, which may be a polar molecule such as a protein (~2-nm diameter) or a colloidal bead (~20-nm diameter). If a single acyl chain is attached, it may be able to insert into the lipid bilayer to its fullest extent. The phase preference that it will show will then depend upon the length and degree of unsaturation of the acyl chain. If a second chain is attached to the particle at some distance on its surface from the first one, the curvature of the particle surface may prevent either of the chains from fully inserting into the membrane, thereby making their effective lengths shorter than they actually are. There may therefore be a tendency for such a biliganded particle to partition into a more disordered phase. Alternatively, if the hydrophobic association energy is adequate for the purpose, there will be a tendency for the curved particle to deform (depress) the planar bilayer surface, resulting in a preference for its association with a more plastic (disordered) phase. This hypothesis is illustrated in Fig. 5 (*top*). The degree to which the chains are extracted from the membrane (or the membrane surface is depressed or deformed) is dependent upon the separation distance between the attachment sites of the chains and the radius of the globular particle, as illustrated

TABLE 3 Fluorescence emission anisotropy of NBD-labeled amphiphiles in liquid ordered and liquid disordered phases at 30°C

	Fluorescence emission anisotropy		
	l_d phase	l_o phase	Phase coexist.
NBD-di$C_{18:0}$PE	0.079	0.128	0.113
NBD-$C_{16:0}C_{18:1\text{-(cis)}}$PE	0.076	0.121	0.098

The l_d-phase bilayers were prepared from pure DMPC, the l_o-phase bilayers were prepared from a 65:35 molar ratio mixture of DMPC and cholesterol, and the bilayers with phase coexistence were prepared from a 82.5:17.5 molar mixture of DMPC and cholesterol, which, at 30°C, has about equal mass fractions of l_d and l_o phases.

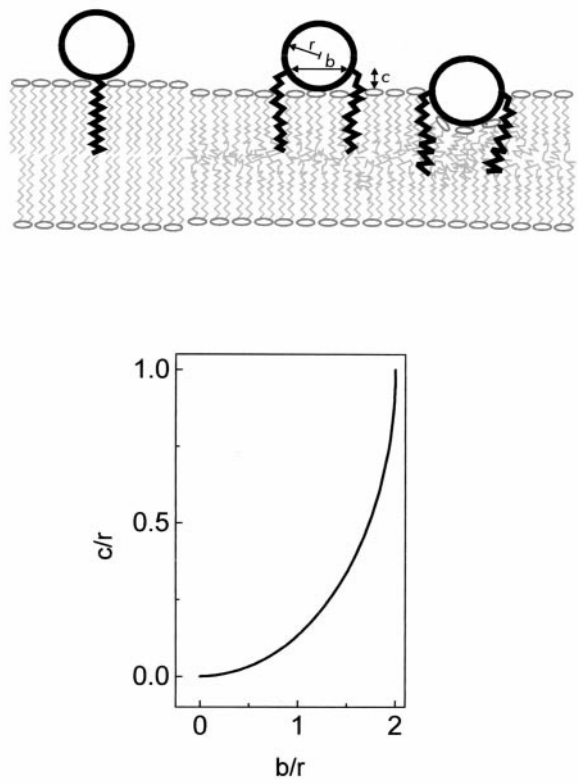

FIGURE 5 (*Top*) A hypothesis for the behavior of a polar globular particle anchored by one and two acyl chains to a membrane. Two cases are illustrated. *Left*: Attachment by a single long chain that may cause the particle to partition into a more ordered phase. *Right*: Attachment by two chains that, depending upon the separation distance, *b*, from their mutual attachment sites to the particle, may either cause the chains to be drawn out of the membrane by a certain length, *c*, or cause the particle to sink into the membrane surface. (*Bottom*) Dependence of the length by which the chains are drawn out of the membrane, *c*, upon the separation distance between chain attachment sites, *b*. In this figure, *r* is the radius of the globular particle.

in the bottom of Fig. 5. Considering that the maximum separation of chain attachment sites is the diameter of the particle, the maximum length by which the chain insertion in the membrane is reduced is the radius of the particle. It may be recalled that the length of a fully extended $C_{18:0}$ chain is ~2.5 nm, which is also the diameter of a globular protein molecule, with a molecular mass of ~25,000 Da, and is an order of magnitude smaller than a typical colloidal gold bead.

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