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Orientational distribution of linear dye molecules in bilayer membranes

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Abstract

The fluorescence decay of the linear dye probe DODCI in bilayer membranes is faster in the presence of sucrose in aqueous solution. This is a consequence of the effect of refractive index on the radiative rate of the oriented molecules inside the bilayer. The second-rank order parameter $\langle P_2(\cos\theta)\rangle$, determined by the lifetime method, is consistently less than that determined by the anisotropy method. This discrepancy is satisfactorily explained by a bimodal orientational distribution whereas an unimodal distribution is not adequate.

1. Introduction

Organic fluorescent dyes are commonly employed to understand the structure and dynamics of biological membranes in relation to membrane related functions [1–4]. Most of these organic molecules are lipophilic in nature and are preferentially solubilized in lipid bilayer membranes. The site of solubilization and its local structure (orientational distribution) in the membrane must be known for the unambiguous interpretation of membrane-based biologically important phenomena; for example, the molecular mechanism for the action of drugs and potential sensing dyes [5–7]. The anisotropic nature of the biological membrane restricts the orientational distribution of these molecules in the lipid bilayer. Several physical techniques can be used to identify the molecule in the membrane and determine the partition coefficient. However, the site of solubilization of the organic molecule and its local structure, namely the orientational distribution, are not easily determined. NMR techniques cannot be used for this purpose at the biological concentration (micromolar) limits and at the dye to lipid ratio (preferentially less than 1:500) where the membrane structure will not get disturbed by the extraneous dye. Diphenyl hexatriene (DPH) is one of the commonly used fluorescent probe molecules for investigating the properties of bilayer membranes. Because of its structure, it is easily solubilized in the membrane phase and its location in the membrane and the orientational distribution has been the subject of numerous studies [3,8–22].

The question of the orientational distribution of the linear dye molecule DPH in a bilayer membrane has been addressed in several experimental studies using fluorescence anisotropy as the measured parameter [3,8–22]. A fixed orientation for the dye in the membrane was ruled out by the low value of steady-state anisotropy and more importantly the ani-

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sotropy decay. Many models of orientational distribution were considered based on empirical (Gaussian model [10]) and theoretical (Maier–Saupe model [8]) considerations. Yet another relevant issue was concerned with the number of distinct populations of the dye in the membrane. For example, a single population is represented by a unimodal orientation distribution whereas a bimodal distribution would indicate two populations. The bimodal distribution is preferred over the unimodal distribution to interpret the fluorescence anisotropy decay kinetics of DPH in vesicles, planar bilayer membranes and in lipid monolayers [9–13,15,23,24]. A recent Monte Carlo dynamics simulation of hydrophobic probe molecules in bilayer membranes gives support for the two population model [25]. The fluorescence lifetime of a linear dye in the bilayer membrane is also sensitive to its orientation and this is only recently used to address the question of orientation of the dye [26].

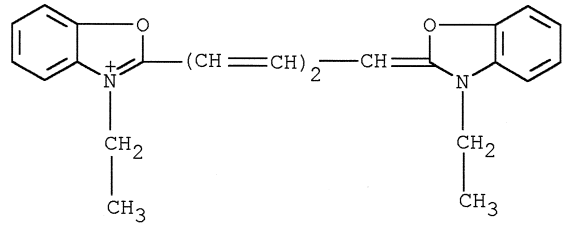
The fluorescence decay of a linear dye molecule intercalated in a lipid bilayer membrane is faster in presence of sucrose in aqueous solution [26,27]. This is a consequence of the effect of refractive index on the radiative decay of an oriented molecule. The fluorescence lifetime of a dye molecule in a bilayer membrane depends on its orientation in the membrane and the refractive indices of the aqueous and lipid media. This can be used to deduce the orientation of the dye molecule in terms of the order parameters. After extensive analysis of fluorescence lifetime and anisotropy experimental results, Toptygin and Brand concluded that the order parameters determined from the lifetime results do not match with those determined from the anisotropy measurements [26]. The discrepancy could not be satisfactorily resolved and hence the question of the orientation of DPH in a DPPC bilayer membrane was left as an open question [26,14].

In addition to DPH, dicarbocyanines are the only organic molecules which show the effect of refractive index on the lifetime of oriented dye molecules [27,28]. In this Letter, we report that the discrepancy between the two order parameters determined from lifetime and anisotropy measurements observed in the case of DPH and in the case of another linear dye DODCI can be consistently explained by the model of bimodal orientational distribution of the dye in a membrane.

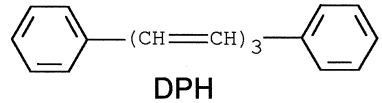
2. Materials and methods

Fluorescence measurements were carried out on DODCI (3,3′-diethyloxadicarbocyanine iodide; Exciton, USA) incorporated in egg PC (L-α-phosphatidyl choline from fresh egg yolk, Sigma Chemical, USA), DLPC (1,2-dilauryl phosphatidyl choline, Sigma Chemical) and DPPC (1,2-dipalmitoyl phosphatidyl choline, Sigma Chemical) vesicles. Fig. 1 shows the structure of the dyes. The sonicated egg PC liposomes were prepared in pH 7.4 or 4.3 buffer (10 mM CH_3COONa, 10 mM NaH_2PO_4, 10 mM MES (2[*N*-morpholino]ethanesulfonic acid) and 150 mM NaCl) as described in Ref. [29]. Sucrose was used to vary the refractive index of the aqueous medium. Increasing the sucrose concentration from 0% to 44.4% (w/w) increased the refractive index from 1.334 to 1.413. The lipid concentration used in these experiments was ~ 0.1 mg/ml (~ 0.14 mM). The dyes were added from the stock solutions made in ethanol to the vesicles and kept overnight. The final samples contain $< 1\%$ v/v ethanol. The dye to lipid ratio was kept approximately at 1:500. All measurements were carried out in air-saturated solutions at room temperature (25°C).

The steady-state fluorescence intensity and anisotropy measurements were made using either a Shimadzu RF540 or SPEX Fluorolog 1681 T format spectrofluorophotometer. The time-resolved fluorescence measurements were made using a high repeti-



DODCI



DPH

Fig. 1. Chemical structures of DODCI and DPH.

tion rate (800 kHz) picosecond dye laser (Rhodamine 6G) coupled with a time-correlated single-photon counting (TCSPC) arrangement described elsewhere [30], using a microchannel plate photomultiplier (Hamamatsu 2809). The time-resolved fluorescence intensity and anisotropy measurements were made at long wavelengths of excitation and emission (580 and 690 nm) to avoid the contribution from the dye present in water, as described before [27]. The sample was excited with vertically polarized light and the fluorescence decay was collected with the emission polarizer kept at the magic angle ($\sim 54.7°$) with respect to the excitation polarizer for measuring lifetimes. For the anisotropy measurements, the fluorescence intensity decays were measured with the emission polarizer set at parallel or perpendicular orientation with respect to the excitation polarizer. The geometry factor (G-factor) for the TCSPC arrangement was determined by the tail matching method using the dye solution in ethanol for which the rotational correlation time (0.25 ns) is much faster than its fluorescence lifetime (1.07 ns). The instrument response function (IRF) was recorded using a non-dairy creamer scattering solution. The full width at half maximum (FWHM) of the IRF is ~ 200 ps. A typical peak count in the emission decay for fluorescence intensity and anisotropy measurements was $\sim 10\,000$.

The experimentally measured fluorescence decay data, $F(t)$, is a convolution of the instrument response function, $R(t)$, with the intensity decay function, $I(t)$:

$$F(t) = \int_0^t R(s)\, I(t-s)\, \mathrm{d}s . \tag{1}$$

In discrete exponential analysis, the intensity decay function is represented as a normalized multi-exponential:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) , \tag{2}$$

where α_i and τ_i are the amplitudes (with $\sum_i \alpha_i = 1$) and the lifetimes, respectively. The parameters of the decay function $I(t)$, namely α_i and τ_i, were determined using experimentally determined $F(t)$ and $R(t)$, respectively, by an iterative deconvolution procedure using the Levenberg–Marquardt algorithm for optimization of the parameters [30–32]. The goodness of fit of experimental $F(t)$ and calculated $F(t)$ is judged by the χ^2 value (close to 1) and the random residual distribution.

The time-resolved anisotropy decay $r(t)$ was calculated using the parallel ($I_{\parallel}(t)$) and perpendicular polarized ($I_{\perp}(t)$) intensity decays as

$$r(t) = \frac{I_{\parallel} - GI_{\perp}}{I_{\parallel} + 2GI_{\perp}} , \tag{3}$$

where G is the G-factor for the TCSPC spectrometer. The polarized intensity decays $I_{\parallel}(t)$ and $I_{\perp}(t)$ were obtained by a deconvolution procedure from the experimentally measured polarized fluorescence decays $F_{\parallel}(t)$ and $F_{\perp}(t)$, respectively, with the instrument response function $R(t)$.

2.1. Determination of order parameters

2.1.1. Order parameter from lifetime measurements

The radiative rate of a linear fluorescent molecule in a bilayer membrane is given by Eq. (4) [33,26].

$$k_{\mathrm{r}} = \frac{4\omega^3}{3\hbar c^3} f^2 |\mu|^2 n_0 \left(\sin^2\theta + \frac{n_0^4}{n_1^4} \cos^2\theta \right) , \tag{4}$$

where ω is the circular frequency of fluorescence light, $\hbar$ is Planck's constant, c is the speed of light, f is a factor which accounts for the difference between the local electric field experienced by the dye and the macroscopic field inside the layer, μ is the matrix element of the emission electric dipole operator, n_0 and n_1 are the refractive indices of the aqueous medium present on both sides of the bilayer and the bilayer, respectively, and θ is the angle between the molecular emission dipole and the normal to the surface of the bilayer. Eq. (4) is based on an isotropic refractive index (n_1) for the bilayer membrane. The effect of anisotropy in the index of refraction (birefringence) of the bilayer, if any, is assumed to be negligible. If k_{nr} is the nonradiative rate (experimentally determined) then fluorescence lifetime is $(k_{\mathrm{r}} + k_{\mathrm{nr}})^{-1}$. The fluorescence decay of the molecule is faster (lifetime decreases as a result of the increase in the radiative rate) with the increase in the refractive index of the aqueous solution which is experimentally observed for DPH and a few other linear dicarbocyanine dyes [26–28]. If $P(\theta)$ is the orientational distribution of the linear molecules with

respect to the membrane normal the second-rank order parameter, $\langle P_2(\cos\theta)\rangle_\tau$ can be determined from the variation of $k_r(=\tau^{-1}-k_{nr})$ with n_0 and from Eq. (4) [26].

$$\langle P_2(\cos\theta)\rangle_\tau = \frac{An_1^4 - \frac{1}{2}B}{An_1^4 + B}, \quad (5)$$

where A and B are the slope and intercept, respectively, of the linear plot

$$\frac{\left(\frac{1}{\tau} - k_{nr}\right)}{n_0} = A\left(n_0^4\right) + B. \quad (6)$$

The subscript τ indicates that the order parameter is determined from the lifetime data.

The refractive index of the membrane is determined as described in Ref. [26]. The light scattering by the vesicles depends on the difference between the refractive indices of the membrane (n_1) and the external medium (n_0). The scattering will be minimum when the two refractive indices are equal. In the vicinity of $n_0 = n_1$, the scattering efficiency can be described by the parabola

$$A = \alpha(n_0 - n_1)^2 + \beta, \quad (7)$$

where A is the absorbence of unlabelled vesicles, and α and β are constants. The refractive index of the membrane (n_1) is determined by fitting the absorbence to the above parabolic equation.

2.1.2. Order parameter from anisotropy measurements

The second-rank order parameter can also be determined from the values of anisotropy at infinite time (r_∞) and the anisotropy value at zero time (r_0) using the equation

$$\langle P_2(\cos\theta)\rangle_a = (r_\infty/r_0)^{1/2}. \quad (8)$$

The subscript a indicates that the order parameter is determined from the anisotropy data. This measures the orientational freedom of the probe population in the bilayer.

3. Results and discussion

The fluorescence decay of DODCI in the three lipid membranes is biexponential with the lifetimes close to 0.68 and 1.76 ns. The short lifetime is associated with the dye in the aqueous phase and/or exposed to the aqueous phase. The long lifetime component is associated with the dye inside the bilayer membrane [27,28]. The fluorescence decay is sensitive to the concentration of sucrose in the aqueous solution. At higher concentrations of sucrose the fluorescence decay is faster which is clearly the effect of increased refractive index on the radiative rate according to Eq. (4). Fig. 2 shows the decrease of the long lifetime component of DODCI in the three lipid membranes egg PC, DLPC and DPPC, as a function of refractive index of the aqueous medium.

The decrease of the fluorescence lifetime of the membrane-bound dye with the refractive index of the aqueous solution is consistent with Eq. (4). For a quantitative estimate of the order parameter, $\langle P_2(\cos\theta)\rangle_\tau$, from the experimental data it is necessary to determine k_{nr}. The calculation of k_{nr} requires

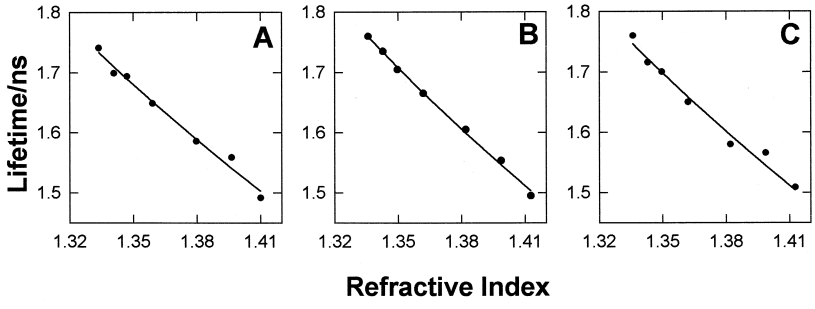


Fig. 2. Variation of long lifetime of DODCI in: (A) egg PC; (B) DLPC; and (C) DPPC vesicles, with the refractive index of the aqueous medium.

the knowledge of the quantum yield of fluorescence for the membrane-bound dye with the long lifetime. In a system where the fluorescence decay is multiexponential and the fluorescence is due to multiple species, it is not possible to determine k_{nr} for a particular species. Hence, the k_{nr} for the membrane-bound species ($\tau = 1.76$ ns) is determined as follows. The radiative rate k_r for the membrane-bound dye is independent of orientation when $n_0 = n_1$ in Eq. (4). Therefore, the value of k_r in the membrane when the refractive index of the aqueous solution is n_1 is taken to be equal to the value of k_r determined in an aqueous glycerol solution whose refractive index is n_1. The value of k_r in aqueous glycerol was determined using the Strickler–Berg equation [34].

$$k_r = 2.88 \times 10^{-9} n_1^2 \int \frac{(2\nu_0 - \nu)^2}{\nu} \varepsilon_\nu \, d\nu, \qquad (9)$$

where n_1 is the refractive index of the medium, ε_ν is the molar extinction coefficient at the frequency ν (in cm^{-1}) and ν_0 is the frequency of S_0–S_1 transition.

The refractive index of the egg PC membrane was calculated as 1.406 according to Eq. (7). Fig. 3 shows the variation of the absorbence of the unlabelled vesicles with the external refractive index, measured at a lipid concentration of 1.43 mg/ml and at the wavelength 450 nm. The radiative rate k_r was determined as 0.513 ns^{-1} following the Strickler–Berg equation (9) using the spectra of DODCI obtained in aqueous glycerol (56% w/w) which has a refractive index of 1.406. The measured fluorescence lifetime for the membrane-bound dye for the same condition is 1.52 ns. The nonradiative rate for the dye is calculated as $(\tau^{-1} - k_r)$.

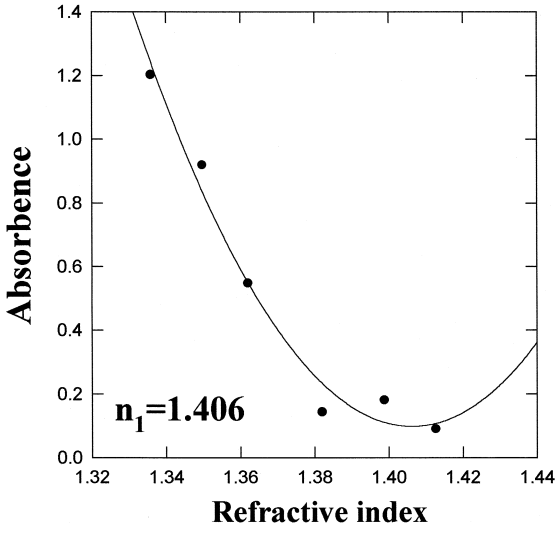


Fig. 3. The plot of 'absorbence' due to scattering of unlabelled vesicles at 450 nm vs. refractive index of the aqueous medium.

The fluorescence lifetime variation of the membrane-bound dye as a function of sucrose concentration in aqueous solution (Fig. 2) was used to calculate the value of $\langle P_2(\cos\theta)\rangle_\tau$ using Eqs. (5) and (6). The calculated value of $\langle P_2(\cos\theta)\rangle_\tau$ from the lifetime results is 0.428 in egg PC. The calculated values of $\langle P_2(\cos\theta)\rangle_\tau$ for DODCI in DPPC and DLPC are 0.435 and 0.568, respectively.

The order parameter for the dye bound to the membrane was also calculated from the fluorescence anisotropy measurements using Eq. (8). The value of the anisotropy at zero time (r_0) is characteristic of the dye molecules and the excitation and emission wavelengths. The r_0 value was determined to be 0.375 from the time-resolved anisotropy decay of DODCI in glycerol in which the rotational correlation time of the dye is very long. The anisotropy at infinite time (r_∞) was determined to be 0.099 from the time-resolved anisotropy decay of DODCI in egg PC membrane. It may be noted that fluorescence decay at times greater than 2 ns is strongly weighted towards the longest lifetime component (in this case the membrane-bound dye with lifetime of 1.76 ns) and hence the experimentally determined r_∞ value is associated with the same species. The value of $\langle P_2(\cos\theta)\rangle_a$ calculated using Eq. (8) is 0.513 in egg PC. The values of $\langle P_2(\cos\theta)\rangle_a$ for the other membranes DPPC and DLPC are 0.628 and 0.688, respectively.

It is clear that the values of $\langle P_2(\cos\theta)\rangle_\tau$ and $\langle P_2(\cos\theta)\rangle_a$ are not equal. This disagreement is similar to that observed by Toptygin and Brand for DPH in DPPC [26]. In the case of DPH in DPPC at 20°C, the values of the order parameters $\langle P_2(\cos\theta)\rangle_\tau$ and $\langle P_2(\cos\theta)\rangle_a$ were 0.285 and 0.91, respectively [26]. $\langle P_2(\cos\theta)\rangle_a$ and $\langle P_2(\cos\theta)\rangle_\tau$ are expected to be equal only if the orientational distribution of the linear dye is unimodal with the director axis of the orientational distribution coinciding with the membrane normal. Thus, it is necessary to examine other

orientational distribution models which predict $\langle P_2(\cos\theta)\rangle_\tau$ to be different from that of $\langle P_2(\cos\theta)\rangle_a$.

3.1. Unimodal distribution model

In this model it is assumed that the population of membrane-bound dye molecules associated with the long lifetime is orientationally distributed in the membrane as a single population about a local director axis. Toptygin and Brand [26] argued that if the director axis is parallel to the membrane normal then $\langle P_2(\cos\theta)\rangle_\tau$ and $\langle P_2(\cos\theta)\rangle_a$ ought to be quantitatively identical. However, if the director axis is tilted by θ' from the membrane normal then,

$$\left\langle P_2(\cos\theta)\right\rangle_\tau = \left\langle P_2(\cos\theta')\right\rangle \times \left\langle P_2(\cos\theta)\right\rangle_a . \tag{10}$$

The application of this model by Toptygin and Brand [26] to the experimental results of DPH in DPPC led to the result that $\theta' = 43°$. It was speculated that the orientation of the acyl chains of the lipid are tilted from the normal and thus the linear dye molecules are also aligned in the same direction as the lipid molecules. Application of this model to our experimental results of different cyanine dyes in egg PC membrane gave dye-dependent orientation angles [27]. The calculated value of θ' for DODCI in different membranes are as follows: 19° for egg PC, 27° for DPPC and 20° for DLPC. It is unlikely that the orientation of the lipid chain would change with the dye molecule for a lipid to dye ratio of 500:1. The model of unimodal distribution with a tilted director axis is therefore not valid.

3.2. Bimodal distribution model

In this model, it is assumed that the molecules are distributed as two populations which interconvert very slowly compared to the intra-population molecular dynamics. The simplest model is the case where the director axis of one population is oriented along the membrane normal and that of the other population is oriented parallel to the membrane. The usefulness of this model to explain the discrepancy between $\langle P_2(\cos\theta)\rangle_a$ and $\langle P_2(\cos\theta)\rangle_\tau$ has been examined.

The bimodal orientational distribution $P(\theta)$ is considered as a sum of two orientational populations, one centered about the membrane normal $P'(\theta)$ and the other parallel to the membrane surface $P''(\theta)$.

$$P(\theta) = P'(\theta) + P''(\theta), \tag{11}$$

where θ is the angle measured with respect to the membrane normal with the normalization condition:

$$\int_0^\pi P(\theta)\sin\theta\,\mathrm{d}\theta = 1. \tag{12}$$

$\langle P_2(\cos\theta)\rangle_\tau$ is calculated with respect to the membrane normal as

$$\left\langle P_2(\cos\theta)\right\rangle_\tau = \int_0^\pi P_2(\cos\theta)\,P(\theta)\sin\theta\,\mathrm{d}\theta. \tag{13}$$

$\langle P_2(\cos\theta)\rangle_a$ is calculated by taking into account the fact that the director axes for the two distributions are mutually perpendicular.

$$\left\langle P_2(\cos\theta)\right\rangle_a = \int_0^\pi \left[P'(\theta)\,P_2(\cos\theta) + P''(\theta) \times P_2\left(\cos(\pi/2-\theta)\right)\right]\sin\theta\,\mathrm{d}\theta. \tag{14}$$

For simplicity, the two distribution functions $P'(\theta)$ and $P''(\theta)$ are considered as Gaussians centered about the membrane normal and perpendicular to the membrane normal [10] as

$$P'(\theta) = a_1 \exp\left(-\frac{\theta^2}{w_1^2}\right), \tag{15a}$$

$$P''(\theta) = a_2 \exp\left(-\frac{\left(\frac{1}{2}\pi-\theta\right)^2}{w_2^2}\right), \tag{15b}$$

where a_1 and a_2 are the amplitudes and w_1 and w_2 are the widths of the two Gaussians. The normalization condition (Eq. (12)) requires that only three of these four parameters are independent.

The parameters of the double Gaussian (DG) distribution function $P(\theta)$, namely the amplitudes a_1, a_2 and widths w_1, w_2, were determined by an iterative method [32] for which $\langle P_2(\cos\theta)\rangle_a$ and $\langle P_2(\cos\theta)\rangle_\tau$ are in quantitative agreement. For this purpose, an initial set of parameters was assumed and it was ascertained that the final optimized values

Table 1
Bimodal orientational distributions for DODCI and DPH in different bilayer membranes

System	$\langle P_2 \rangle_\tau$	$\langle P_2 \rangle_a$	Model	w_1 / λ_1 [a]	w_2 / λ_2 [a]	a_1	a_2	f_1	f_2
DODCI + egg PC	0.428	0.513	DG	40.3 ± 0.1	61.9 ± 8.1	0.0691 ± 0.0002	0.0024 ± 0.0001	0.8985 ± 0.0083	0.1015 ± 0.0083
pH 7.4, 25°C			BRD	2.26 ± 0.02	3.18 ± 0.46	0.0092 ± 0.0001	0.0006 ± 0.0001	0.9287 ± 0.0025	0.0713 ± 0.0025
DODCI + DPPC	0.435	0.628	DG	33.5 ± 0.9	52.7 ± 14.0	0.0849 ± 0.0007	0.0057 ± 0.0001	0.7842 ± 0.0340	0.2158 ± 0.0340
pH 4.3, 25°C			BRD	2.83 ± 0.01	5.41 ± 1.10	0.0006 ± 0.0001	0.0006 ± 0.0003	0.8527 ± 0.0045	0.1473 ± 0.0045
DODCI + DLPC	0.568	0.688	DG	28.7 ± 0.6	57.0 ± 6.6	0.1242 ± 0.0024	0.0036 ± 0.0002	0.8551 ± 0.0183	0.1449 ± 0.0183
pH 4.3, 25°C			BRD	3.51 ± 0.01	5.89 ± 1.40	0.0043 ± 0.0000	0.0003 ± 0.0001	0.9094 ± 0.0025	0.0906 ± 0.0025
DPH + DPPC	0.285[b]	0.91[b]	DG	15.7 ± 0.7	20.7 ± 1.3	0.2559 ± 0.0221	0.0255 ± 0.0013	0.5374 ± 0.0036	0.4626 ± 0.0036
pH 7.4, 20°C			BRD	8.19 ± 0.26	11.04 ± 1.50	0.0001 ± 0.0000	0.0002 ± 0.0001	0.5581 ± 0.0032	0.4419 ± 0.0032

The two order parameters $\langle P_2 \rangle_\tau$ and $\langle P_2 \rangle_a$ are determined from lifetime and anisotropy methods, respectively; the optimized parameters (see text) for the double Gaussian (DG) and Brownian rotational diffusion (BRD) models and the two fractional amplitudes f_1 and f_2 in the two populations.
[a] w_1, w_2 in the case of DG, and λ_1, λ_2 in the case of BRD model.
[b] Result from Ref. [26].

are independent of initial values of the parameters. The optimized values of the amplitudes and the widths, an average of ten trials, for the different experimental systems are given in Table 1. The fractions f_1 and f_2 of the two populations are also given in Table 1. Fig. 4 shows the orientational distribution $P(\theta)$ and the population distribution $P(\theta)\sin\theta$ for these cases.

The results described above suggest that a bimodal orientational distribution of dye molecules in membranes explains satisfactorily the discrepancy between the two experimental order parameters. However, the Gaussian distribution of orientations which is assumed in the above calculations may not be strictly valid. A more reasonable distribution function is based on the Brownian rotational diffusion model (BRD) where the molecule is moving in an effective potential created by the lipid molecules in the system [8]. The dye probes in the two populations are considered to experience two different orienting potentials [35]. In the simplest case (Maier–Saupe model [8]), one can write

$$P'(\theta) = a_1 \exp(\lambda_1 P_2(\cos\theta)), \tag{16a}$$

$$P''(\theta) = a_2 \exp(-\lambda_2 P_2(\cos\theta)), \tag{16b}$$

where a_1 and a_2 are the amplitudes and $\lambda_1(>0)$ and $\lambda_2(>0)$ are the adjustable parameters consistent with the normalization condition (Eq. (12)). As described above, these parameters were optimized using an iterative method [32] for which the two order parameters match. The optimized values which are independent of the initial values (ten trials) for the different experimental systems are given in Table 1. The orientational distribution function $P(\theta)$ and the population distribution function $P(\theta)\sin\theta$ are shown in Fig. 4.

Thus, the discrepancy between the two order parameters from lifetime and anisotropy measurements

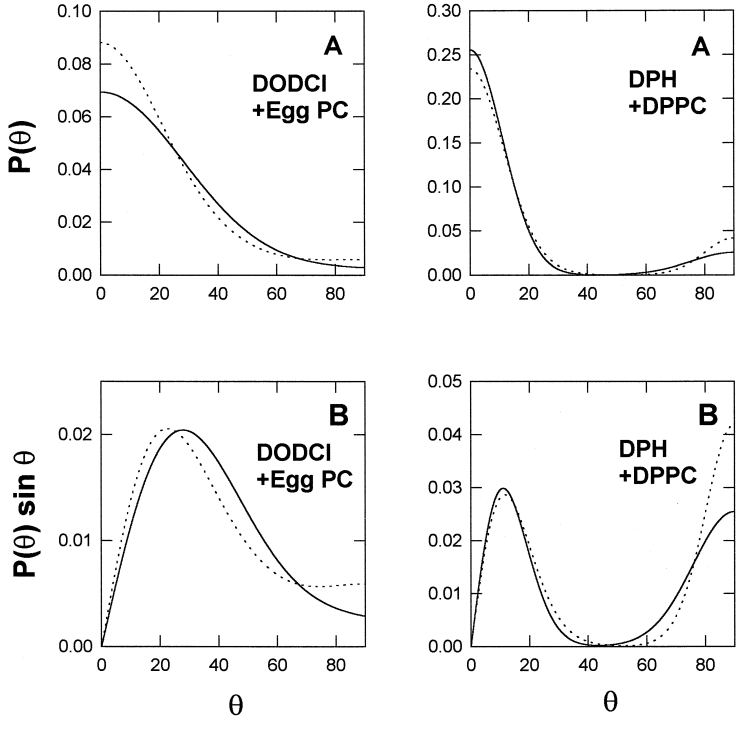


Fig. 4. (A) Bimodal orientational distribution functions, $P(\theta)$, and (B) population distribution functions, $P(\theta)\sin\theta$ for DODCI in egg PC and DPH in DPPC. The solid line shows the functions for double Gaussian (DG) and the dotted line shows the functions for the Brownian rotational diffusion (BRD) model (see text).

is satisfactorily explained by the bimodal distribution model. However, the fractions of populations oriented parallel and perpendicular to the membrane and the shape of the population distribution depends upon the assumed functions for $P(\theta)$.

It may be noted that $P(\theta)$ gives the orientational probability distribution whereas $P(\theta)\sin\theta$ gives the actual number density of the dye molecules. Hence the number density appears significantly different from the orientational probability distribution. In the case of DODCI in egg PC, the population distribution appears nearly unimodal with a peak at $\theta = 26°$ because of large widths (Table 1) associated with the two populations. On the other hand, the population distribution for DPH in DPPC is unambiguously bimodal with peaks at $\theta = 12°$ and $\theta = \pi/2$.

The results and analysis presented in this Letter use fluorescence lifetime data in addition to the conventional fluorescence anisotropy data to deduce the orientational distribution of linear dye molecules in a bilayer membrane. It is shown that the experimental results are consistent with a bimodal orientational distribution and not a unimodal distribution.

4. Summary

The fluorescence decay of linear dye probes such as DODCI and DPH intercalated in bilayer membranes is sensitive to the presence of sucrose in aqueous solution which is a consequence of the effect of refractive index on the radiative rate of the oriented dye molecules. The second-rank order parameters for the oriented dye molecules were determined by two independent methods. The values of the two order parameters are not in agreement. It is shown that a bimodal orientational distribution can satisfactorily explain the discrepancy whereas an unimodal orientational distribution is not adequate.

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