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ANGLE RESOLVED FLUORESCENCE DEPOLARISATION OF 1,6-DIPHENYL-1,3,5 HEXATRIENE (DPH) IN UNSATURATED DIGALACTOSYL DIACYLGLYCEROL (DGDG) LIPID BILAYERS

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The orientational ordering and dynamics of DPH molecules embedded in macroscopically ordered bilayers of the unsaturated galactolipid digalactosyl diacylglycerol (DGDG) were studied by angle-resolved fluorescence depolarisation at room temperature.

The depolarisation ratios exhibited a weaker angular dependence than those observed for DPH incorporated in multibilayers of saturated lecithins above their phase transition. The results reflect the differences in the modes of reorientational motion of the DPH molecules in these systems while a similar orientational order is found. It is suggested that DPH molecules in bilayers of DGDG cannot be considered to possess an effective cylindrical symmetry in contrast to DPH in bilayers of saturated lecithins.

Angle-resolved fluorescence depolarisation (AFD) experiments for studying the macroscopic molecular order and dynamics of fluorescent porobes such as 1,6-diphenyl-1,3,5-hexatriene (DPH) incorporated into macroscopically ordered multibilayers of saturated lipids with and without cholesterol were recently described [1,2]. The analysis of the experimental data is carried out on assuming the DPH probes to possess an effective cylindrical symmetry about their long axes. This can only be justified if the reorientation of the long molecular axes of the molecules is much slower than the rotation about these axes.

Here we report an application of the AFD method to DPH molecules embedded in bilayers of unsaturated lipids. The angular dependence of

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the depolarisation ratios appears to be significantly different from those observed for saturated bilayers [2]. As in the latter systems we have found different order parameters S_μ and S_ν for the absorption and emission moments respectively. Since the absorption moment lies parallel to the long molecular axis, this indicates that the emission moment is tilted relative to that axis. Because of this molecular property, the fluorescence depolarisation experiment senses the reorientation of the DPH molecules around their long axes. Unsaturation appears to have a strong influence on the reorientational dynamics of the probes, particularly lowering the rate of motion around the long axes. We conclude that DPH molecules should not be used as probes to monitor ordering and dynamics of bilayers of unsaturated lipids.

MATERIALS AND METHODS

Digalactosyl diacylglycerol (DGDG) was prepared from spinach leaves [3,4]. The fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) was purchased from Aldrich. Ethanol (AR) from Baker and doubly distilled water were used. Macroscopically oriented bilayers were prepared as described previously [2]. To prevent oxidation of the DGDG samples all preparations were carried out strictly under a nitrogen atmosphere. No oxidation of the lipids could be detected on recording their UV absorption spectra [5].

Angle-resolved fluorescence depolarisation (AFD) experiments were carried out on a home-built fluorimeter [2]. The necessary precautions to minimize systematic errors in AFD experiments have been fully discussed in [2]. DPH was excited at 365 nm with a full width at half maximum (FWHM) bandwidth of 8 nm. The fluorescence was observed at room temperature (25°C) by means of interference filters, FWHM 12 nm, at 432 nm. The intrinsic fluorescence of well-aligned pure lipid samples was less than 1% of the fluorescence of well-aligned DPH-containing samples. No photo-bleaching of the probe molecules under our experimental conditions was observed. The depolarisation ratios were measured as a function of two angles θ and ϕ which, respectively, describe the directions of the excitation and observation relative to the normal of the lipid bilayers. The five parameters R_i, $i = 1 \text{—} 5$, determining the angle dependence, were obtained as described in [1,2]. These contain all the information about the orientational order and dynamics of the DPH probes.

RESULTS

Different samples yielded depolarisation ratios reproducible to an accuracy of 2%. On rotating the samples through an arbitrary angle about the normal to their surfaces the same ratios were found

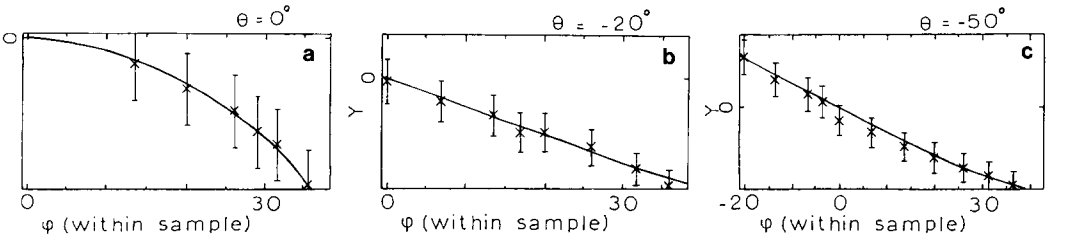

Fig. 1 Theoretical fits of AFD results for a DGDG/water 70/30 (w/w) bilayer system with 4 x 10^{-3} M DPH at 25°C.

* : experimental values, rel. accuracy 2%. Solid lines indicate least squares fits according to [2].

a), b), c) "extraordinary" experiments. Vertical scales θ= 0° : 0.23; θ = -20° : 0.47 and θ = -50° : 0.61.

within experimental error. This indicates that the DPH molecules possess a uniaxially symmetric macroscopic distribution in the multibilayers. Results, corrected for refraction and transmission losses [2], are given in Figs. 1a-c. These figures show $Y(\theta,\phi) = A(\theta)\sin^2\phi + B(\theta)\sin 2\phi$, [2], as a function of ϕ for several values of θ. Note that the experimental errors (2%) appear larger for lower values of θ. $Y(\theta,\phi)$ are scaled differently for each angle.

The values of the parameters R_i, i = 1 - 5 obtained from the experimental data following [2] are shown in Table I under A. It can be clearly seen that $R_2 \neq R_3$, so that the emission moment of DPH is tilted relative to the long molecular axis [2]. The order parameter of the absorption moment, and hence that of the long axis, $S_\mu = \langle P_2 \rangle = 0.26$, was found to be considerably higher than that for the emission moment, $S_\nu = 0.04$. This yields an angle of tilt $\beta_{\mu\nu} \sim 49^\circ$. However, a tilt angle $\beta_{\mu\nu} \sim 39^\circ$ is obtained from an analysis of the time-averaged correlation functions, Table II. The latter value agrees well with those found for DPH molecules in bilayers of saturated lecithins above their phase transition [2].

The information about $\langle P_4 \rangle$ and the reorientational dynamics is contained in the time-averaged correlation functions g_0, g_1 and g_2 [2]. We have interpreted these functions in terms of the strong-collision

Table I

EVALUATION OF R_1---R_5 FOR ORIENTED LIPID MEMBRANE SYSTEMS
(70/30 (w/w) DGDG/water mixture with $4x10^{-3}$ M DPH)

	R_1	R_2	R_3	R_4	R_5
A	0.230	-0.339	0.451	0.306	1.81
B	0.20	-0.19	0.41	0.23	1.9
C	0.21	-0.19	0.43	0.23	1.9
D	0.25	-0.03	0.28	0.47	1.3

A. Values obtained from a non-linear least-squares fit of the data.

B. Best fit values for the strong collision model.

C. Best fit for the stochastic rotational diffusion model.

D. Best fit for the "Wobbling-in-cone" model.

(SC) model [2,6], the stochastic rotational diffusion (SRD) model [6-8] and the "wobbling-in-cone" model [9,10], and the results are shown in Table II. In the analysis we have fitted the free model parameters to the R_i's by a non-linear least squares procedure. The best fits are shown in Table I.

Table II

EVALUATION OF PARAMETERS OBTAINED FOR THREE MODELS
(70/30 (w/w) DGDG/WATER MIXTURE WITH $4x10^{-3}$ M DPH)

	$\langle P_2 \rangle$	$\langle P_4 \rangle$	W_0	τ_0^* (nsec)	$P_2(\cos\beta_\nu)$	$D_\perp \tau_F$	$D_W \tau_F$
A	0.26	0.23	>0.95	$\gg 10$	0.38		
B	0.26	0.23			0.39	$\ll 1$	
C	0.18	-0.11			0.49		$\ll 1$

*τ_F = 10 nsec [12].

A. Strong collision model.

B. Stochastic rotational diffusion model.

C. "Wobbling-in-cone" model.

All three models yield the surprising result that the reorientational motion of the long molecular axes is extremely slow on the time scale defined by the fluorescence life time τ_F. Consequently, the correlation functions $G_0(t)$, $G_1(t)$, and $G_2(t)$ [2] do not decay appreciably during this time. The time-averaged correlation functions determined by our experiment are thus given by the values of the G_k's, k = 0,1,2, at t = 0 and are strictly model independent [2,6,8,10]. They can be expressed solely in terms of $\langle P_2 \rangle$ and $\langle P_4 \rangle$ [6,10]. Inspection of Table II shows that the SC and SRD models yield similar values for the order parameters. On the other hand, the "wobbling-in-cone" model yields a negative value for $\langle P_4 \rangle$. This is an inherent feature of the model which predicts that $\langle P_4 \rangle \leq 0$ for $0 \leq \langle P_2 \rangle \leq 0.55$ [10,11]. Clearly, the latter model provides an unsatisfactory description of the orientational order.

The results strongly indicate that the assumption of an effective cylindrical symmetry for the DPH molecules is inconsistent with the experimental data for DGDG bilayers. This in contrast to the behaviour in bilayers of saturated lecithins [2]. We suggest that this is due to a slowing down of the reorientational motion around the long molecular axes of the DPH molecules in bilayers of unsaturated lipids. As a complete analysis of the results, including non-cylindrically symmetric behaviour, is fairly complicated and requires the use of many model parameters [1,6], we conclude that DPH molecules are unsuitable probes for bilayers of unsaturated lipids.

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