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Slow-motion ESR study of order and dynamics in oriented lipid multibilayers: effects of unsaturation and hydration

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Electron spin resonance (ESR) experiments were carried out on 3-doxyl-5α-cholestane spin-label (CSL) molecules embedded in macroscopically oriented multibilayers of dimyristoylphosphatidylcholine (DMPC), palmitoyloleoylphosphatidylcholine (POPC), dioleoylphosphatidylcholine (DOPC) and dilinoleoylphosphatidylcholine (DLPC). For these lipids we studied the effects of temperature, hydration and unsaturation on the orientational order parameters and rotational motions of the probe molecules in the liquid crystalline phase. The experimental ESR spectra were simulated by a numerical solution of the stochastic Liouville equation (SLE) for the density matrix of a spin-label molecule. This allows extraction of detailed information about both molecular order and rotational dynamics. The data show that, in our temperature range, the lipid systems are in the slow-motion regime, thereby precluding a motional narrowing interpretation. This is illustrated by a simple model calculation which shows that a fast-motion interpretation seriously overestimates the order parameters. We have compared our results with data obtained independently from angle-resolved fluorescence depolarization (AFD) experiments on oriented bilayers in which 1-[4-(trimethylammonio)phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH) molecules were used as fluorescent probes (Deinum et al., (1988) Biochemistry 27, 852–860). It is found that the orientational order and the rotational dynamics obtained with both techniques agree well. This shows that the probe molecules do not perturb the local bilayer structure to any large extent and that they indeed reflect the intrinsic behaviour of the lipid molecules. Upon increase in temperature or hydration, we observe faster reorientational motion and lower molecular ordering. In contrast, we do not find any systematic effect of unsaturation on molecular reorientational motion. Our results indicate that changes in membrane molecular order and reorientational dynamics have to be considered separately and are not necessarily correlated as implied by the common concept of membrane fluidity.

Introduction

Much of our knowledge of the structure and dynamics of biological membranes has been obtained from the study of the properties of model systems, such as lipid bilayers arranged in vesicles or planar structures.

In the liquid crystalline phase, the lipid molecules are known to lie with their long axes preferentially along the normal to the bilayer surface and to undergo rapid lateral diffusion [1]. In view of the strongly anisotropic behaviour of the lipid molecules, it is necessary to include both their orientational order parameters and their rotational diffusion coefficients in any description of the physical properties of the model membranes. These quantities can be obtained from ^{2}H- and ^{13}C-NMR, ESR and FD studies. The latter techniques, however, monitor the behaviour of extraneous probe molecules introduced into the bilayer structure at small concentrations. The behaviour of the probe molecules is taken to reflect that of the surrounding lipids.

An important aspect which determines the information content of the experimental technique is its intrinsic timescale, τ_{int}. In magnetic resonance techniques, this is determined by the anisotropy of the magnetic interactions (about 10^{-5} s for ^{2}H-NMR, versus 10^{-9} s

Abbreviations: CSL, 4′,4′-dimethylspiro[5α-cholestane-3,2′-oxazolidin]-3′-yloxy spin-label; AFD, angle-resolved fluorescence depolarization; SLE, stochastic Liouville equation; DMPC, dimyristoylphosphatidylcholine; DLPC, dilinoleoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; POPC, palmitoyloleoylphosphatidylcholine; TMA-DPH, 1-[4-(trimethylammonio)phenyl]-6-phenyl-1,3,5-hexatriene.

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for ESR), and in FD experiments by the lifetime of the excited state (about 10^{-9} s).

Optimally, the rotational motions, characterized by a rotational correlation time τ_R, should be studied by a technique for which $\tau_R \approx \tau_{int}$. Since in membrane systems $\tau_R \approx 10^{-10}$–10^{-7} s, ESR and FD techniques are particularly suitable for studying the dynamics of the lipid molecules.

Since $\tau_r \approx \tau_{int}$ for ESR, the interpretation of experimental data requires a theoretical approach different from that for NMR, where $\tau_R \ll \tau_{int}$. While the latter can be analyzed in the framework of the Redfield Motional Narrowing Approximation [2], the ESR spectral lineshapes in the slow-motion regime ($10^{-9} < \tau_R < 10^{-7}$ s) must be described in terms of the SLE formalism [3–6]. Indeed, it has been shown that the two techniques yield a consistent picture of the orientational order and rotational dynamics in the bilayer system, provided the differences in the approach to the analysis are recognized [6,7].

Unfortunately, the SLE approach to the analysis of the ESR lineshapes is cumbersome and requires the application of complex numerical spectral simulation techniques. Consequently, most studies have made use of results based on the assumption of motional narrowing. This assumption, as will be shown here, is not generally valid for lipid bilayer systems in the liquid crystalline phase.

In this work, we employ the nitroxide spin-label, CSL. The nitroxide group of CSL is rigidly bound to the nucleus of the molecule. This is of crucial importance for the interpretation of the ESR spectra, as the order and dynamics of the CSL molecule as a whole are directly reflected in the spectral lineshapes. As CSL is a spin-label with a large A-tensor anisotropy, the ESR spectra are optimally sensitive to anisotropy in the rotational motions of the molecule [8].

We shall show here that the orientational order and the rotational dynamics obtained with CSL as a probe molecule agree well with data obtained independently from AFD experiments. This shows that the probe molecules do not perturb the local bilayer structure to any large extent and that they indeed reflect the intrinsic behaviour of the lipid molecules.

One of the topics in lipid research is the study of the effects of unsaturated bonds in the hydrocarbon chains on orientational order and rotational motions of the lipid molecules. Increased unsaturation is known to reduce the order parameters [9–11]. The effect of unsaturation on molecular motion, however, is not so well understood. Earlier studies have reported small increases in molecular mobility upon introduction of unsaturated bonds in liposomes [10]. However, recent AFD studies on macroscopically oriented samples [11] have reported that increased unsaturation caused a reduction of both the order parameters and the reorientational dynamics of the probe molecules. This was felt to conflict with current concepts of membrane fluidity.

The hydration of the lipid headgroups is also an important factor determining the behaviour of the lipid molecules. It is known from X-ray diffraction studies that the area per lipid molecule at the bilayer surface increases when the hydration rate is increased [12,13]. However, only a few studies of the effect of hydration on molecular order and dynamics have been reported [14]. Furthermore, little is known about the effects of increasing insaturation at different hydration of the lipid headgroups.

We have therefore carried out a comprehensive ESR study of the effects of unsaturation and hydration on the order and dynamics of oriented bilayers of phosphatidylcholines with CSL probe molecules. These experiments were carried out at various temperatures, $T = 19$–$45\,^{\circ}$C, in the liquid crystalline phase. The ESR spectra were analyzed in terms of the SLE, affording an analysis over the whole dynamic range from very slow motions ($\tau_R \approx 10^{-6}$ s) to very fast motions ($\tau_R \ll 10^{-9}$ s). In this way, the order parameters and rotational diffusion coefficients can be obtained from the experimental spectra. Our data show that motions in the oriented systems fall in the slow-motion regime, thus precluding a motional narrowing interpretation.

The reorientational motions are found to increase on increase in temperature or hydration. A concomitant reduction in the molecular order in the lipid bilayer system is observed. Furthermore, the introduction of unsaturated bonds reduces the molecular ordering. In contrast, we do not find any systematic effect of unsaturation on molecular reorientational motion. This is in contrast with previous results and supports the suggestion that a clear distinction must be made between molecular orientational order and reorientational dynamics [11,15].

Theory

In ESR experiments, the observed quantity is the absorbed microwave energy. The molecular orientations and the molecular dynamics of the ESR probe molecules are reflected in the shape of the absorption spectrum. We can calculate the spectral lineshapes from the magnetization of the sample, which is found from a statistical average over the magnetic moments of the probe molecules. This average can be evaluated from the spin-density matrix, which gives the statistical weights of the various spin states at a given time. The general theory of the lineshapes has been described in detail elsewhere [3,5,6,16] and will only be summarized here.

The time-evolution of the spin-label density matrix $\rho(\Omega, t)$ in the presence of an oscillating microwave field

is described by the SLE:

$$\partial/\partial t\, \rho(\Omega, t) = -i[\mathscr{H}(\Omega) + \epsilon(t), \rho(\Omega, t)] - \Gamma(\rho(\Omega, t) - \rho_0(\Omega)) \tag{1}$$

where the Euler angles, Ω, specify the orientation of the spin-label molecule. The time-independent part $\mathscr{H}(\Omega)$ of the one-particle Hamiltonian is taken to be the sum of the electronic Zeeman and hyperfine structure term:

$$\mathscr{H}(\Omega) = (\beta_e/h)\vec{H}_0 \cdot \vec{g}(\Omega) \cdot \vec{S} - \gamma_e \vec{I} \cdot \vec{A}(\Omega) \cdot \vec{S} \tag{2}$$

where $\vec{H}_0$ is the external static magnetic field and $\vec{S}$ and $\vec{I}$ are the electron and nuclear spin operators, respectively. We quantize these operators along the direction of $\vec{H}_0$. The small nuclear Zeeman term is neglected in Eqn. 2. Moreover, we omit the nonsecular terms (that is, terms proportional to S_x or S_y) from the Hamiltonian (Eqn. 2), since their effect on the spectrum is negligible [17].

The microwave field $H_x(t) = 2H_1 \cos\omega t$ is applied perpendicular to the external $\vec{H}_0$ field, and gives rise to the Hamiltonian $\epsilon(t)$:

$$\epsilon(t) = 1/2\ \gamma_e H_1 (S_+ e^{-i\omega t} + S_- e^{+i\omega t}) \tag{3}$$

where $S_\pm = S_x \pm iS_y$ are the spin-raising and -lowering operators. The operator, Γ, induces relaxation towards the equilibrium density matrix $\rho_0(\Omega)$ and is given by:

$$\Gamma = \Gamma_\Omega + \Gamma_R \tag{4a}$$

where Γ_R is a phenomenological operator introducing inhomogeneous line broadening into the spectrum. Its operation on a spin 1/2 density matrix is defined as:

$$\Gamma_R \begin{pmatrix} \rho_{11} & \rho_{12} \\ \rho_{21} & \rho_{22} \end{pmatrix} = \begin{pmatrix} 0 & \dfrac{-\rho_{12}}{T_2} \\ \dfrac{-\rho_{21}}{T_2} & 0 \end{pmatrix} \tag{4b}$$

The rotational diffusion operator, Γ_Ω, describes the conserved flow of the probability density $f(\Omega, t)$ of finding a particular orientation, Ω, at time, t:

$$\mathrm{d}/\mathrm{d}t\, f(\Omega, t) = \partial/\partial t f(\Omega, t) + \Gamma_\Omega f(\Omega, t) = 0 \tag{5}$$

We take the rotational diffusion to be a Brownian diffusion process in the presence of an external potential, $U(\Omega)$ [3,18,19]:

$$\Gamma_\Omega = \vec{M} \cdot \vec{\vec{D}}[\vec{M} + 1/kT(\vec{M}U(\Omega))] \tag{6}$$

where $\vec{M}$ and $\vec{\vec{D}}$ are the angular momentum operator and the (anisotropic) diffusion tensor, respectively. The scalar potential, $U(\Omega)$, phenomenologically describes the ordering effects of the surrounding lipid molecules on the nitroxide spin-label molecule. It is taken to be cylindrically symmetric around the director axis, that is:

$$U(\Omega) = U(\beta) = -kT\{\lambda_2 P_2(\cos\beta) + \lambda_4 P_4(\cos\beta)\} \tag{7a}$$

and depends only on the angle β between the long axis of the spin-probe and the normal to the bilayer surface. P_2 and P_4 denote Legendre polynomials of order 2 and 4:

$$P_2(\cos\beta) = 1/2(3\cos^2\beta - 1) \tag{7b}$$

$$P_4(\cos\beta) = 1/8(35\cos^4\beta - 30\cos^2\beta + 3) \tag{7c}$$

The normalized angular equilibrium distribution $\hat{f}_0(\Omega)$ for $U(\Omega)$ is given by:

$$\hat{f}_0(\Omega) = \frac{f_0(\beta)}{4\pi^2} = \frac{1}{4\pi^2} \cdot \frac{\exp(-U(\beta)/kT)}{\int_0^\beta \exp(-U(\beta)/kT)\sin\beta\, \mathrm{d}\beta} \tag{8a}$$

with

$$\Gamma_\Omega \hat{f}_0(\Omega) = 0 \tag{8b}$$

We take the equilibrium density matrix, $\rho_0(\Omega)$, to be [20]:

$$\rho_0(\Omega) = \hat{f}_0(\Omega)\left(1 - h\frac{H(\Omega)}{kT}\right) \Big/ Tr\left(1 - h\frac{H(\Omega)}{kT}\right) \tag{9}$$

In ESR experiments, the observed quantity is the absorbed energy, P. In view of the inhomogeneous line broadening observed in CW ESR, this absorbed energy $P(\omega)$ is assumed to be the Gaussian accumulation of power absorption $P_i(\omega)$ from individual spin packages:

$$P(\omega) = \int_{-\infty}^{\infty} \mathrm{d}\omega' \frac{1}{\sigma_G\sqrt{2\pi}} \exp\left[-\omega'^2/2\sigma_G^2\right] P_i(\omega + \omega') \tag{10}$$

where the Gaussian broadening, σ_G, is a measure of variation of the local static magnetic field. In comparison with the Lorentzian broadening introduced via the relaxation operator, Γ_R, the effect of this Gaussian broadening is less pronounced in the wings of the absorption lines. Apart from this detail, T_2 and σ_G have largely similar effects and are interchangeable in this sense. A fit in the absence of Gaussian broadening would result in lower values of T_2 with essentially identical values of diffusion rates and order parameters.

Per cycle and unit volume, P_i is given by [21]:

$$P_i(\omega) = \frac{\omega}{2\pi} \int_0^{2\pi/\omega} -M_x(t) \frac{\mathrm{d}H_x(t)}{\mathrm{d}t} \mathrm{d}t \tag{11}$$

The magnetization density, $M_x(t)$ can be evaluated from the solution $\rho(\Omega, t)$ of Eqn. 1:

$$M_x(t) = nh\gamma_e \int_\Omega Tr[\rho(\Omega, t) S_x] \hat{f}_0(\Omega)\, \mathrm{d}\Omega \tag{12}$$

where n stands for the spin-label density.

The SLE can be solved by a variety of methods [22–26]. We have employed the method of Freed and co-workers [27], where the solution of $\rho(\Omega, t)$ is found by a Laplace transformation of the time-variable to frequency space and the decomposition of the angular dependence in Wigner rotation matrices $D^L_{mn}(\Omega)$. The resulting matrix equation can be efficiently solved by application of the Lanczos algorithm [27,28]. Numerically stable results were obtained with the following subset of Wigner rotation matrices: $0 \leqslant L \leqslant 10$, L even; $-4 \leqslant n \leqslant 4$, n even; and $-2 \leqslant m \leqslant 2$.

To gain insight into the merits of the procedure described above it is instructive to compare the values for the order parameter $\langle P_2 \rangle$ obtained from the SLE solution with values extracted from a motional narrowing analysis. For a given, realistic set of parameters, we stimulated spectra for several values of the potential parameter λ_2 (with $\lambda_4 = 0$) over a wide range of diffusion rates. The exact result $\langle P_2 \rangle_e$ depends on the potential parameter λ_2 only, and is given by the average of the second-order Legendre polynomial, $P_2(\cos \beta)$ as:

$$\langle P_2 \rangle_e = \int_{-1}^{1} \mathrm{d} \cos \beta \, f_0(\beta) \, P_2(\cos \beta) \tag{13}$$

In contrast, the motional narrowing expression $\langle P_2 \rangle_{MN}$ is given by [29]:

$$\langle P_2 \rangle_{MN} = \frac{A_{iso} - A_e}{A_{iso} - A_{zz}} \tag{14}$$

Here A_{zz} is the z component of the hyperfine tensor used in the simulations. $A_{\parallel}$ and $A_{iso} = 1/3(A_{\parallel} + 2A_{\perp})$ are found from the line-splittings $A_{\parallel}$ and $A_{\perp}$ of the simulated spectra at $\theta = 0$ and 90°, respectively. Here θ is the angle between the macroscopic director, $\vec{n}$, of the sample and the static magnetic field, $\vec{H}_0$. Fig. 1 clearly shows that the motional narrowing assumption is accurate for large rotational diffusion coefficients, D:

$$\lim_{D \to \infty} \langle P_2 \rangle_{MN} = \langle P_2 \rangle_e \tag{15}$$

but seriously overestimates the molecular order for slow diffusion rates ($D < 10^8$ rad$^2 \cdot$ s^{-1}). As expected, this effect is more pronounced in systems with low molecular ordering. Clearly, the motional narrowing interpretation cannot be used for the lipid bilayer systems under study, where we find $\langle P_2 \rangle \approx 0.4$–$0.7$ and $D \approx 10^7$–10^8 rad$^2 \cdot$ s^{-1}.

Finally, we note that the order parameter, $\langle P_4 \rangle$, is defined as:

$$\langle P_4 \rangle = \int_{-1}^{1} \mathrm{d} \cos \beta \, f_0(\beta) \, P_4(\cos \beta) \tag{16}$$

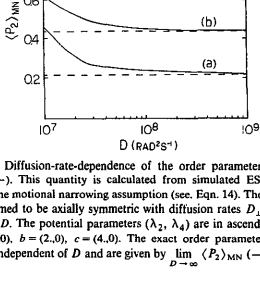

Fig. 1. Diffusion-rate-dependence of the order parameter $\langle P_2 \rangle_{MN}$ (———). This quantity is calculated from simulated ESR spectra using the motional narrowing assumption (see. Eqn. 14). The molecule is assumed to be axially symmetric with diffusion rates $D_\perp = D$ and $D_\parallel = 5D$. The potential parameters (λ_2, λ_4) are in ascending order: $a = (1.,0)$, $b = (2.,0)$, $c = (4.,0)$. The exact order parameters, $\langle P_2 \rangle_e$, are independent of D and are given by $\lim_{D \to \infty} \langle P_2 \rangle_{MN}$ (— — —).

Materials and Methods

DMPC, POPC, DOPC, and DLPC were purchased from Sigma (St. Louis, MO, U.S.A.) and used without further purification. The spin-label CSL was bought from Aldrich Chemical Company (Milwaukee, WI, U.S.A.). The purity of the lipids and the spin-label was checked when necessary by high-performance thin-layer chromatography (HPTLC).

In all our experiments, a CSL concentration of 1 mol% was used. At this concentration, the spin–spin interactions between individual CSL molecules are expected [30] to produce homogeneous line broadening of approx. 0.5 G. This value is comfortably exceeded by the combined effect of Lorentzian and Gaussian broadening of approx 1.5 G.

Sample preparation

The lipid/CSL mixtures were prepared by dissolving the components in chloroform. After mixing, the chloroform was removed under vacuum. Equilibration over a saturated K_2SO_4 solution provided lipid/CSL mixtures with a water content of 24 wt%. Samples with 12 wt% were derived from this by subsequent equilibration over a saturated sodium acetate (CH_3COONa) solution. The equilibration time was longer than 16 h. The resulting water concentration was determined gravimetrically with an estimated relative uncertainty of 10%. The hydrated lipid material was oriented between glass plates ($0.2 \times 4 \times 8$ mm) by application of shear pressure, leading to stacks of about a thousand bilayers. The macroscopic alignment was checked optically with a polarizing microscope equipped with a first-order red plate. Four to six individual samples were stacked in order to improve the signal-to-noise ratio.

The samples were kept in the dark under a nitrogen atmosphere as much as possible. The polyunsaturated lipids, DOPC and DLPC, were manipulated strictly under nitrogen atmosphere to avoid oxidation of the unsaturated bonds. In spite of the fact that the method of preparation could not completely eliminate the presence of oxygen, even DLPC samples had signal losses of less than 5% over a period of 4 days, indicating a negligibly slow oxidation of the unsaturated bonds.

ESR experiments

ESR experiments were carried out using a Varian E-9 X-band spectrometer equipped with a TM110 cavity. The sample was placed in a quartz tube above a saturated salt solution to maintain the water concentration and in a nitrogen atmosphere to prevent oxidation. The salt solution, at the bottom of the tube, was placed well away from the active region of the cavity. The sample temperature was regulated within 1°C with a Varian V4540 variable temperature accessory and measured by a copper-constantan thermocouple placed above the sample, just outside the active region of the cavity. The orientation of the sample director relative to the applied static magnetic field was varied using a home-built goniometer with an accuracy of ±1°. ESR spectra were recorded at a microwave power level of 1–2 mW, well below saturation. A magnetic field modulation of 1 G (top–top) with a frequency of 100 kHz was used to detect the derivative of the absorption signal. The background ESR signal, arising from the quartz tube and the glass plates, was subtracted from the measurements before analysis.

Results and Discussion

Experiments on oriented lipid multibilayer systems of DMPC, POPC, DOPC and DLPC were carried out in the temperature range of 35–45°C for DMPC and 19–45°C for the other lipids, well above the gel-liquid crystalline phase transition, and at two different water concentrations of 12 and 24 wt%.

The paramagnetic probe molecule used throughout was CSL. This is a rigid molecule of well-known geometrical structure [31]. In the liquid crystalline phase, the average orientation of the long axis of the CSL molecule is perpendicular to the bilayer plane [32–34]. The rigidity allows identification of the orientation of the paramagnetic centre with that of the probe as a whole, which is of crucial importance for the interpretation of the ESR spectra [35]. In view of the geometrical form of the molecule, its rotational diffusion in the membrane is assumed to be cylindrically symmetric, with a rotational diffusion tensor of the form $\bar{\bar{D}} = \mathrm{diag}(D_\perp, D_\perp, D_\parallel)$, where $D_\parallel$ and $D_\perp$ are the diffusion rates for rotation around the long molecular axis and rotation of that axis, respectively. The principal axes of the magnetic tensors, $\bar{\bar{g}}$ and $\bar{\bar{A}}$, are assumed to coincide and have a diagonal form in the CSL reference frame. The right-handed reference frame is chosen with the x axis oriented along the N–O bond, the y axis oriented along the $2p$-π orbital of the nitrogen and the z axis oriented along the long molecular axis.

Approximate values of the hyperfine constants were obtained from the simulation of rigid-limit spectra of ordered systems at −25°C. In the liquid crystalline phase the quality of the simulations of experimental spectra was significantly improved on using slightly adjusted values (within 5%) of the hyperfine constants. For DMPC, POPC and DOPC, good fits were obtained with $\bar{\bar{A}} = \mathrm{diag}\ (5.6,\ 34.0,\ 5.3)$. For DLPC, however, these hyperfine constants did not yield satisfactory fits, and $\bar{\bar{A}} = \mathrm{diag}(5{,}6,\ 32.4,\ 5.6)$ was used instead. We feel that this adjustment might be related to the tendency for phase separation in DLPC (see below). In contrast, the gyromagnetic tensor $\bar{\bar{g}}$ was obtained from Dammers, Ref. 16, and kept unchanged throughout the simulations: $\bar{\bar{g}} = \mathrm{diag}(2.0081,\ 2.0024,\ 2.0061)$. Similarly, the isotropic spin–spin relaxation time $T_2 = 2 \cdot 10^{-7}$ s was taken to be constant.

With this procedure, the adjustable parameters used for fitting were the rotational diffusion coefficients, $D_\parallel$ and $D_\perp$; the parameters, λ_2 and λ_4, of the ordering potential; and the Gaussian broadening, σ_G. For every sample and temperature ESR spectra were recorded at four different orientations of the sample, i.e., $\theta = 0$, 30, 60 and 90°, respectively, where θ is the angle between the macroscopic director to the sample and the static magnetic field, $\vec{H}_0$. This procedure avoids the ambiguities reported previously [36] which arise from the fit of the spectrum at only a single orientation. The sensitivity of the fits was improved by simultaneous fitting of spectra at intermediate angles, $\theta = 30$ and 60°C, and, in particular, the value of λ_4 could be determined more accurately. The quality of a fit was judged visually.

We estimate that the values obtained of the model parameters are reliable within the following bounds: λ_2 5%, λ_4 30%, $D_\parallel$ 30%, $D_\perp$ 25% and σ_G 10%. The fitting parameters were found to be essentially independent of each other: changing one of them could not be compensated by a variation of the other parameters. The quality of a representative simulation can be estimated from Fig. 2.

The temperature-dependence of the ordering and dynamics of the CSL probe molecules in the different lipid systems at a water concentration of 24 wt% are summarized in Table I. All the lipid systems studied here showed an increase of the diffusion parameters, $D_\parallel$ and $D_\perp$, by a factor of 2–4 on increasing the temperature from 20 to 45°C. This relatively strong temperature-dependence confirms earlier results [6,37,38]. Moreover, the order of magnitude of $D_\parallel$ and $D_\perp$ indicates that a motional narrowing analysis of

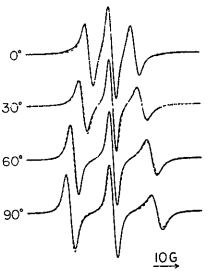

Fig. 2. Experimental (———) and simulated (– – – —) ESR spectra of CSL in DOPC multibilayers for various angles between the static magnetic field and the director to the bilayer surface at a resonance frequency of 9.0 GHz. The temperature is 35 °C, the water content 24 wt%. The fitting parameters are: $D_{\parallel} = 3 \cdot 10^8$ rad$^2 \cdot$s^{-1}, $D_{\perp} = 4 \cdot 10^7$ rad$^2 \cdot$s^{-1}, $\lambda_2 = 2.3$, $\lambda_4 = 0.1$ and $\sigma_G = 1.0$ G.

these spectra is not justified. Experiments on fully hydrated oriented lipid multibilayer systems [6] and on vesicle- and liposome systems (Korstanje et al., unpublished data) show that the slow-motion analysis is appropriate to all the fluid phases of bilayer systems and is not simply a consequence of the hydration levels used here.

For the ratio $N = D_{\parallel}/D_{\perp}$, we find values which are within a factor of 3 of the value of $N = 4.7$ expected from the geometry of the CSL molecule [39]. Comparable values of N for CSL in lipid bilayers were reported by Schindler and Seelig [40] and Meirovitch and Freed [41]. However, substantially larger values for N have also been reported [42,43]. We have found that high values of N, $N \approx 40$–50, are compatible with an analysis of the spectrum at a single orientation, i.e., $\theta = 0°$. This is in agreement with Koole et al. [36]. However, if the spectra obtained at other sample orientations are included in the analysis, then substantially lower N values are needed to provide satisfactory fits. We conclude that reliable values of the diffusion constants can only be obtained from angle-resolved ESR experiments.

Turning to the order parameters, we find a gradual decrease of $\langle P_2 \rangle$ and $\langle P_4 \rangle$ with increasing temperature. Qualitatively, the same picture arises at the lower water concentration of 12 wt%, an exception being DLPC. The latter displays a phase separation into ordered and disordered domains at this low water concentration, thus precluding an interpretation of such ESR spectra within our current model.

The order parameters and diffusion coefficients obtained here from ESR experiments can be compared with previous results from AFD experiments on oriented bilayers [11], in which TMA-DPH molecules were used as fluorescent probes. These molecules behave analogously to the CSL molecules as they are also expected to be anchored between the polar headgroups of the phospholipids [44]. The AFD experiments were analyzed with the same diffusion mechanism for reorientational motion so that a direct comparison of both the order parameters and the diffusion coefficients could be made. As shown in Table II, the agreement between the two approaches is satisfactory for the order parameters, and within the estimated uncertainties for the diffusion rates. (Note, however, that only the diffusion coefficient $D_{\perp}$ can be determined for the TMA-DPH molecules.) It indicates that, in spite of their vastly different chemical composition, both types of probe molecule undergo similar interactions with the surrounding lipid matrix.

Table III shows the effect of hydration on the parameters determined from our ESR experiments. An in-

TABLE I

Best fit parameters for molecular ordering and dynamics of CSL in oriented multibilayers of different lipids with 24 wt% of water

N is defined as $D_{\parallel}/D_{\perp}$, while $\langle P_2 \rangle$ and $\langle P_4 \rangle$ were calculated from λ_2 and λ_4 using Eqns. 13 and 16. The quality of the fits was judged visually. Estimated uncertainties: σ_G 10%, λ_2 5%, λ_4 30%, $D_{\parallel}$ 30% and $D_{\perp}$ 25%.

Lipid	T (°C)	σ_G (G)	λ_2	λ_4	$D_{\parallel}$ (10^8 rad$^2 \cdot$s^{-1})	$D_{\perp}$ (10^8 rad$^2 \cdot$s^{-1})	N	$\langle P_2 \rangle$	$\langle P_4 \rangle$
DMPC	35	1.3	3.3	0.0	2.0	0.34	5.9	0.64	0.28
	45	1.2	2.9	0.0	5.0	0.90	5.6	0.59	0.24
POPC	19	1.4	3.2	0.0	1.2	0.17	7.1	0.63	0.27
	35	1.2	3.0	0.0	2.5	0.45	5.6	0.61	0.25
	45	1.1	2.8	0.0	3.5	0.60	5.8	0.58	0.22
DOPC	21	1.1	2.5	0.0	1.5	0.23	6.5	0.52	0.18
	35	1.0	2.3	0.1	3.0	0.40	7.5	0.51	0.18
	45	1.0	2.0	0.2	5.0	0.50	10	0.46	0.16
DLPC	21	1.0	2.1	0.3	1.4	0.20	7.0	0.50	0.20
	35	0.9	1.8	0.4	2.4	0.28	8.6	0.45	0.18
	45	0.8	1.6	0.5	3.5	0.35	10	0.41	0.17

TABLE II

Comparison of results from ESR and AFD experiments on oriented lipid multibilayers

The results from the AFD experiments, using TMA-DPH as a probe molecule, were obtained from Deinum et al. [11]. The water concentration was 24 wt% throughout. (Comparison with $D_{\parallel}$ is not possible, since the latter cannot be obtained from the AFD experiments.)

Lipid	Experiment	T (°C)	λ_2	λ_4	$D_{\perp}$ (10^7 rad^2·s^{-1})	$\langle P_2 \rangle$	$\langle P_4 \rangle$
DMPC	ESR	35	3.3	0.0	0.34	0.64	0.28
DMPC	AFD	35	3.22	0.07	0.34	0.64	0.29
POPC	ESR	19	3.2	0.0	0.17	0.63	0.27
POPC	AFD	21	2.96	0.53	0.12	0.66	0.35
DOPC	ESR	21	2.5	0.0	0.23	0.52	0.18
DOPC	AFD	21	2.41	0.18	0.28	0.54	0.21
DLPC	ESR	21	2.1	0.3	0.20	0.50	0.20
DLPC	AFD	21	2.18	0.73	0.22	0.57	0.29

crease in the water content induces a decrease in the ordering of the sample and increases the mobility of the probe. Similar trends were observed in AFD experiments on egg PC multibilayers [14].

Similarly, an increase in the unsaturation of the hydrocarbon chains of the lipids results in a clear decrease in the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ (see Fig. 3). This trend is found under all our experimental conditions, in agreement with previous studies [9,38]. We find no evidence for an anomalously low ordering of DOPC bilayers, as reported from AFD studies with DPH fluorescent probes [11].

In marked contrast with the effects of temperature and hydration, we find no systematic effect of increasing unsaturation on the rotational diffusion rates. This is corroborated by recent experiments in vesicle- and liposome systems (Korstanje et al., unpublished data), and conflicts with the recent results of Kusumi and Pasenkiewicz-Gierula [45]. These authors have reported strong effects of alkyl chain length and unsaturation on the diffusion constants obtained from a fast-motion analysis of liposome systems.

Our results indicate that changes in membrane

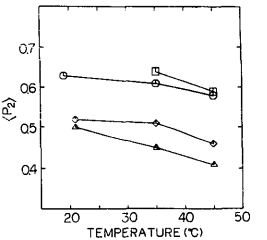

Fig. 3. Temperature-dependence of $\langle P_2 \rangle$ for lipids with a varying degree of unsaturation at a hydration rate of 24 wt%. Lipids are: □, DMPC; ○, POPC; ◇, DOPC; △, DLPC.

TABLE III

Effect of hydration on the order and dynamics of CSL in oriented multibilayers of lipids with a varying degree of unsaturation at a temperature of 45 °C

Estimated uncertainties: water concn. 10%, σ_G 10%, λ_2 5%, λ_4 30%, $D_{\parallel}$ 30% and $D_{\perp}$ 25%.

Lipid	Water concn. (wt%)	σ_G (G)	λ_2	λ_4	$D_{\parallel}$ (10^8 rad^2·s^{-1})	$D_{\perp}$ (10^8 rad^2·s^{-1})	N	$\langle P_2 \rangle$	$\langle P_4 \rangle$
DMPC	24	1.2	2.9	0.0	5.0	0.90	5.6	0.59	0.24
	12	1.1	3.6	0.0	2.4	0.16	15	0.68	0.32
POPC	24	1.1	2.8	0.0	3.5	0.60	5.8	0.58	0.22
	12	1.0	3.0	0.0	3.0	0.40	7.5	0.61	0.25
DOPC	24	1.0	2.0	0.2	5.0	0.50	10	0.46	0.16
	12	0.9	1.8	0.5	2.0	0.30	6.7	0.46	0.20
DLPC	24	0.8	1.6	0.5	3.5	0.35	10	0.41	0.17
	12	–	–	–	–	–	–	–	– [a]

[a] DLPC showed a phase separation at the lower water concentration, precluding an interpretation of the ESR spectra within our current model.

molecular order and reorientational dynamics have to be considered separately and are not necessarily correlated, as implied by the concept of membrane fluidity [46,47].

Conclusions

Simulation of CSL spectra has shown that the stochastic Liouville formulation with slow molecular tumbling yields a satisfactory description of CW ESR spectra in phospholipid bilayers in the liquid crystalline phase. This allows us to obtain both the order parameters and the rotational diffusion coefficients of the bilayer systems. From these values it is clear that we are in the slow-motion regime and that a motional narrowing interpretation may not be used for our systems. Moreover, we have shown that reliable values of the diffusion rates can only be obtained by a simultaneous fit of spectra obtained at different orientations of the sample relative to the applied magnetic field.

We have shown that the orientational order and the rotational dynamics obtained with CSL in planar lipid multibilayers agree well with data obtained independently from AFD experiments on planar lipid systems with TMA-DPH molecules as fluorescent probes. This shows that the probe molecules do not perturb the local bilayer structure to any large extent and that they indeed reflect the intrinsic behaviour of the lipid molecules.

For a given phospholipid sample, we confirm the general trend that faster reorientational motion is associated with lower directional order in the system (and vice versa) under changes in temperature and water content.

In contrast to the clear decrease in the order parameters upon an increase of the number of unsaturated bonds, we find no systematic effect on the rotational mobility of the paramagnetic probe molecules in the liquid crystalline phase.

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