Biochimica et Biophysica Acta, 1022 (1990) 155–162
Elsevier

BBAMEM 74747

Effects of steroid molecules on the dynamical structure of dioleoylphosphatidylcholine and digalactosyldiacylglycerol bilayers

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(Received 18 August 1989)

Key words: Steroid; Dioleoylphosphatidylcholine; Digalactosyldiacylglycerol; Cholestane spin label; Order parameter; Dynamics; Spin label

The ESR spectra of cholestane spin labels (CSL) in dioleoylphosphatidylcholine (DOPC) bilayers containing 20 wt% of cholesterol, 7-dehydrocholesterol, β-sitosterol, stigmasterol and lanosterol exhibit a marked similarity, thus indicating that these steroids induced the same effects on the lipid bilayer over the temperature range 21–55 °C. The incorporation of these steroids into the DOPC bilayers enhances the orientational order of the CSL molecules at every temperature studied, but only induces a pronounced slow-down in their rotational motions at temperatures above 35 °C. Similar results were obtained in DOPC/ergosterol multilamellar liposomes, but the changes are now less pronounced than in the other five DOPC/steroid systems. In contrast, the addition of stigmasterol to digalactosyldiacylglycerol (DGDG) bilayers appears to increase the order parameter $\langle P_2 \rangle$, without affecting the diffusion coefficients. Furthermore, the incorporation of 7-dehydrocholesterol to DGDG bilayers causes a large enhancement in the orientational order, but has only a small effect on $D_\perp$ of the CSL molecules. Importantly, this latter effect appears to be independent of temperature. The marked changes in the rates of the rotational motion brought about by the addition of steroids, contrasts with the lack of a significant effect of unsaturation on the bilayer dynamics reported by us previously (Korstanje et al. (1989), Biochim. Biophys. Acta 980, 225–233, and 982, 196–204).

Introduction

Steroids are common constituents of biological membranes and they are known to influence the structural and functional properties of lipid membranes. Much research effort has been devoted to the study of the effects of cholesterol, and it has been shown to affect a variety of membrane properties (for review, see Refs. 1–4). Other steroids have been studied less extensively, but appear to modulate the properties of the membrane in a similar fashion [4,5].

ESR has been shown to be a powerful technique for studying the effects of cholesterol on the orientational order and rotational dynamics in lipid bilayer membranes [6–16]. To this end nitroxide spin-label molecules, either fatty acids or cholestane, are incorporated into the bilayer structure at low concentrations. The nitroxide ESR spectra obtained from these studies have been commonly analyzed under the assumption that the rotational motions are fast on the timescale defined by the hyperfine interactions of the spin labels. This approach has been criticized in the past few years, as it has been shown that this assumption is not justified for lipid systems at physiologically relevant temperatures [17–20]. In general, the dynamics of these lipid systems falls in the slow-motion regime, with rotational correlation times, τ_R, in the range $10^{-9} < \tau_R < 10^{-7}$ s. The ESR spectral lineshapes now have to be described in terms of the stochastic Liouville equation (SLE) formalism [21–23]. This approach is cumbersome, because the order parameters and rates of rotational motion cannot be extracted from the observed spectra in a straightforward way. Rather, the ESR spectra must be simulated numerically.

The SLE formalism has been used in recent studies of the effect of cholesterol on the dynamic order of

Abbreviations: CSL, 3-doxyl-5α-cholestane spin label; DGDG, digalactosyldiacylglycerol; DOPC, dioleoylphosphatidylcholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; FD, fluorescence depolarization; PC, phosphatidylcholine; SLE, stochastic Liouville equation; SUV, small unilamellar vesicle; TMA-DPH, 1-[4-(trimethylammonio)phenyl]-6-phenyl-1,3,5-hexatriene.

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phosphatidylcholine systems [18,24–26]. Here we report an extension of this work to investigations of the properties of lipid bilayers of DOPC containing other physiologically active steroids. The steroid spin label 3-doxyl-5α-cholestane (CSL) was used as a probe molecule. The effects of cholesterol, 7-dehydrocholesterol, β-sitosterol, stigmasterol, lanosterol, ergosterol and estradiol on the dynamical behaviour of DOPC lipid bilayers arranged in aqueous multilamellar dispersions (liposomes) were studied.

We find that the incorporation of 20 wt% of cholesterol, 7-dehydrocholesterol, β-sitosterol, stigmasterol and lanosterol into DOPC bilayers enhances the orientational order of the CSL molecules, but only induces a pronounced slow-down in their rotational motions at temperatures above 35 °C. The marked changes in the rates of the rotational motion brought about by the addition of steroids, contrast with the lack of a significant effect of unsaturation on the bilayer dynamics [19,20].

Recent fluorescence depolarization (FD) studies from our laboratory using 1,6-diphenyl-1,3,5-hexatriene (DPH) molecules as probes, have suggested that the plant steroid stigmasterol affected the molecular order and dynamics in bilayers of phosphatidylcholines and DGDG in opposite ways [27,28]. We have therefore undertaken an ESR study of the behaviour of stigmasterol in the two lipid systems of DOPC and DGDG. Our results confirm the findings that the addition of stigmasterol has a different effect on DGDG than on DOPC bilayers.

Materials and Methods

DOPC and cholesterol were purchased from Sigma Chemical Company (St Louis, MO, U.S.A.). 7-Dehydrocholesterol, stigmasterol and ergosterol were bought from Fluka A.G. (Buchs, Switzerland) and β-sitosterol, lanosterol and estradiol were bought from Merck (Darmstadt, F.R.G.). DGDG was obtained from Lipid Products (Surrey U.K.). The spin label CSL was bought from Aldrich Chemical Company (Milwaukee, WI, U.S.A.). When necessary, the purity of the lipids, the steroids and the spin label was checked by high performance thin-layer chromatography (HPTLC).

In all our experiments we used a steroid concentration of 20 mol%. The CSL concentration was 1 mol% in all the experiments. At this concentration, the spin-spin interactions between individual CSL molecules are exposed to produce homogeneous line broadening of approx. 0.5 G [29]. This value is comfortably exceeded by the combined effect of Lorentzian and Gaussian broadening of approx. 1.5 G.

Sample preparation

Lipid/steroid/CSL multilamellar liposomes were prepared by dissolving the components in chloroform. After mixing, the chloroform was removed by a flow of nitrogen gas and subsequent storage under vacuum for several hours. The lipid/steroid/CSL mixture was hydrated by the addition of a 20 mM Tris buffer (pH 8.0), containing 7.5 μM EDTA. The resulting mixture, containing 2.3 mg lipid/ml buffer, was homogenized with a vortex mixer for several minutes. 20 μl of the hydrated mixture, put in quartz capillary, was used as a sample. All the preparative steps were carried out in the dark under a nitrogen atmosphere as much as possible to avoid oxidation of the lipids. The samples were used in the ESR experiments within 24 h of their preparation.

ESR experiments

ESR experiments were carried out using a Varian E-9 X-band spectrometer, equipped with a TM110 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. ESR spectra were recorded at a microwave power level of 10 mW, well below saturation. A magnetic field modulation of 1.0–1.6 gauss top-top with a frequency of 100 kHz was used to detect the first derivative of the absorption signal. The background ESR signal, arising from the quartz capillary, was subtracted from the measurements before analysis.

Spectral simulations

The simulation of the experimental ESR spectra was carried out by a numerical solution of the SLE for the density matrix of a spin-label molecule. This approach provides spectral simulations for a wide range of rotational correlation times, from very slow motions ($\tau \approx 10^{-6}$ s) to very fast motions ($\tau \ll 10^{-9}$ s) of the CSL molecules in the lipid bilayers. The technical details of the analysis have been described elsewhere [21,23,30–33] and the method will only be summarized below.

The SLE is the equation of motion for the density matrix of a collection of non-interacting spin-label molecules. The effect of the surrounding ordered lipid matrix on the spin-labels enters the SLE in the form of a relaxation term determined by an anisotropic diffusion operator. The solutions of the SLE allow the computation of the magnetization of and power absorption by the spin-labels.

In our case the relaxation term involves the standard rotational diffusion model [34,35], where the molecules are assumed to undergo small-step, correlated, angular excursions subject to the action of an orienting potential $U(\Omega)$ [36]. This potential is taken to be cylindrically symmetric around the normal to the bilayer surface:

$$U(\Omega) = U(\beta) = -kT\{\lambda_2 P_2(\cos\beta) + \lambda_4 P_4(\cos\beta)\} \quad (1)$$

where β is the angle between the long axis of the spin

probe and the local director to the bilayer surface. P_2 and P_4 denote Legendre polynomials of order 2 and 4:

$$P_2(\cos\beta) = 1/2(3\cos^2\beta - 1) \quad (2)$$

$$P_4(\cos\beta) = 1/8(35\cos^4\beta - 30\cos^2\beta + 3) \quad (3)$$

In view of the geometrical form of the CSL molecule, its rotational diffusion in the membrane is assumed to be cylindrically symmetric, with a rotational diffusion tensor of the form $\boldsymbol{D} = \text{diag}\,(D_\perp, D_\perp, D_\parallel)$, where $D_\parallel$ and $D_\perp$ are the diffusion rates for rotations around the long molecular axis and rotation of that axis, respectively. The orientational distribution function $f(\beta)$ is given by:

$$f(\beta) = \frac{\exp(-U(\beta)/kT)}{\int_0^\pi \exp(-U(\beta)/kT)\sin\beta\,d\beta} \quad (4)$$

The orientational order parameter $\langle P_2\rangle$ is given by the average of the second order Legendre polynomial:

$$\langle P_2\rangle = \int_0^\pi f(\beta)P_2(\cos\beta)\sin\beta\,d\beta \quad (5)$$

$\langle P_4\rangle$ is defined analogously:

$$\langle P_4\rangle = \int_0^\pi f(\beta)P_4(\cos\beta)\sin\beta\,d\beta \quad (6)$$

In multilamellar liposome and SUV suspensions, the directors to the lipid bilayers have a random distribution of orientations relative to the static magnetic field $\overline{H}_0$. On a macroscopic scale, the samples exhibit complete orientational disorder. In this case, the observed ESR signal $P(\omega)$ can be considered to be a superposition of spectra of randomly distributed planar bilayer samples:

$$P(\omega) = \text{Im}\,Z(\omega) \quad (7a)$$

$$Z(\omega) = 1/2\int_0^\pi Z(\omega,\theta)\sin\theta\,d\theta \quad (7b)$$

where $Z(\omega, \theta)$ is the response function of a planar bilayer sample oriented at an angle θ relative to $\overline{H}_0$.

For frequencies near resonance, where $Z(\omega, \theta)$ is a strongly peaked function of θ, a straightforward numerical evaluation of Eqn. 7 requires a very fine integration mesh demanding inordinate amounts of cpu time. We have therefore evaluated the integral using a substraction method which presupposes knowledge of $Z(\omega, \theta)$ at only a small number of orientations θ. Details of this procedure are given in Ref. 20.

Additional line broadening is introduced into the spectrum by a Lorentzian broadening, denoted by a relaxation time T_2, and a Gaussian broadening, σ_G.

Results

ESR experiments in the temperature range of 21 to 55 °C, were carried out on aqueous multilamellar dispersions (liposomes) of DOPC, containing 20 mol% cholesterol, 7-dehydrocholesterol, β-sitosterol, stigmasterol, lanosterol, ergosterol or estradiol. These steroids differ in the structure of the branched side-chain and the steroid nucleus. The hormone estradiol contains a second hydroxyl group instead of a side-chain. In addition, experiments on DGDG multilamellar liposomes, with and without 20 mol% 7-dehydrocholesterol or stigmasterol, were carried out.

In all the experiments, CSL was used as a probe molecule. This molecule has a rigid nucleus of well known geometrical structure [37], which allows identification of the orientation of the paramagnetic nitroxide group with that of the probe as a whole. In this way, the ESR spectra yield information about the overall orientation and rigid body motion of the probe molecules. CSL is anchored with its nitroxide group at the lipid/water interface and in the liquid-crystalline phase, the average orientation of the molecule is perpendicular to the bilayer plane [38–40]. The principal axes of the magnetic $\boldsymbol{g}$- and $\boldsymbol{A}$- tensors are assumed to coincide and have a diagonal form in the CSL reference frame. The right handed reference frame is chosen with the z-axis oriented along the long molecular axis of the CSL molecule.

Good fits were obtained with the same hyperfine constants as those used previously for the simulation of macroscopically unoriented lipid bilayer systems [20]:

$\boldsymbol{A}$ = diag(5.6 G, 34.0 G, 5.3 G)

$\boldsymbol{g}$ = diag(2.0081, 2.0024, 2.0061)

The isotropic spin-spin relaxation time, T_2, was fixed at $T_2 = 2 \cdot 10^{-7}$ s, resulting in a Lorentzian broadening of 0.28 G. This is similar to the value used in previous simulations of ESR spectra from CSL molecules embedded in various lipid systems [19,20].

We have previously shown [20] that the isotropic averaging over all possible orientations of the local bilayer normal washes out the details in ESR spectra from multilamellar liposome and SUV configurations. It was shown that this loss of spectral features reduces the sensitivity of the simulations to changes in the free parameters. We have therefore chosen [20] to restrict the number of free parameters entering the simulations in the following way. The value of λ_4 was fixed to be $\lambda_4 = 0.4 \cdot \lambda_2$, resulting in the broadest possible distribution function $f(\beta)$ exhibiting a monotonous decrease between $\beta = 0°$ and $\beta = 90°$. Good fits were obtained with this choice of λ_4. The ratio $N = D_\parallel/D_\perp$, of the rotational diffusion constants was taken to be 5. This value of N is close to the value of 4.7, expected from the geometry of the CSL molecule [41]. This choice yielded good fits of the observed spectra and furthermore, an increase of N to 10 or more clearly resulted in worse fits.

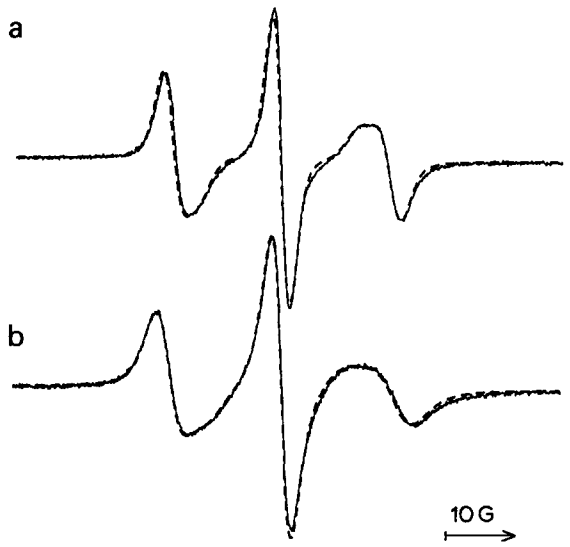

Fig. 1. Experimental (———) and simulated (— — —) ESR spectra of CSL in multilamellar liposomes of DOPC, containing 20 mol% estradiol at (a) 55 °C and (b) 25 °C. The fitting parameters are: (a) $D_\perp = 1.8 \cdot 10^8$ rad$^2 \cdot$s^{-1}, $\lambda_2 = 1.26$ and $\sigma_G = 1.2$ G; (b) $D_\perp = 5.0 \cdot 10^7$ rad$^2 \cdot$s^{-1}, $\lambda_2 = 1.86$ and $\sigma_G = 1.2$ G. Furthermore $D_\parallel = 5D_\perp$ and $\lambda_4 = 0.4\ \lambda_2$.

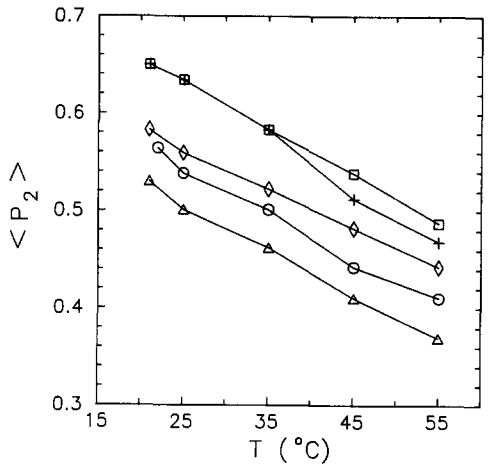

Fig. 2. Temperature dependence of the order parameter $\langle P_2 \rangle$ of CSL molecules embedded in multilamellar liposomes of DOPC with and without 20 mol% steroids. The data for DOPC without steroids were taken from Ref. 20. (○) DOPC without steroids, (□) DOPC with cholesterol, 7-dehydrocholesterol, β-sitosterol or stigmasterol, (+) DOPC with lanosterol, (◇) DOPC with ergosterol, (△) DOPC with estradiol.

This procedure leaves us with three adjustable fitting parameters for spectra of macroscopically unoriented samples: the rotational diffusion coefficient $D_\perp$, the potential parameter λ_2, and the Gaussian broadening σ_G. These fitting parameters are essentially not correlated, the changes in the lineshape caused by varying one of them could not be compensated by changing the values of the other two parameters. We estimate the fitting parameters for liposome and SUV spectra to be reliable within the following bounds: λ_2: 5%, $D_\perp$: 30% and σ_G: 10%. The experimental and simulated spectra of CSL embedded in multilamellar liposomes of DOPC with 20 mol% estradiol, at 25 and 55 °C, are shown in Fig. 1 to illustrate the quality of the fits and the temperature dependence of the spectral lineshapes.

For all experiments it was found that the value of σ_G was between 1.1 and 1.3 G, with an average value of 1.2 G.

DOPC multilamellar liposomes

Incorporation of 20 mol% cholesterol, 7-dehydrocholesterol, β-sitosterol, stigmasterol or lanosterol increased the degree orientational order in DOPC bilayers at every temperature studied (cf. Fig. 2). Interestingly, the effect of ergosterol is smaller than the effect of the other steroids, in particular between 21 and 25 °C.

In marked contrast, we have found the addition of estradiol to cause a decrease in the order. The effect of estradiol on the shape of the orientational distribution function $f(\beta)$ in the lipid bilayer, compared with DOPC and DOPC/cholesterol bilayers, is shown in Fig. 3.

The order parameter $\langle P_2 \rangle$ exhibits an almost linear decrease over the temperature range 21 to 55 °C, Fig. 2. It can be seen that the incorporation of any steroid in the DOPC liposomes essentially only introduces a shift of the $\langle P_2 \rangle$ vs. temperature curve along the $\langle P_2 \rangle$ axis.

The effects of the various steroids on the rotational diffusion coefficient, $D_\perp$, are shown in Fig. 4. Here we find that the change in $D_\perp$ brought about by the steroids to be strongly temperature dependent. At the lower end of the temperature range studied, between 21 and 25 °C, the values of $D_\perp$ are essentially the same, within experimental error, for bilayers with or without steroids. A pronounced reduction in $D_\perp$, compared

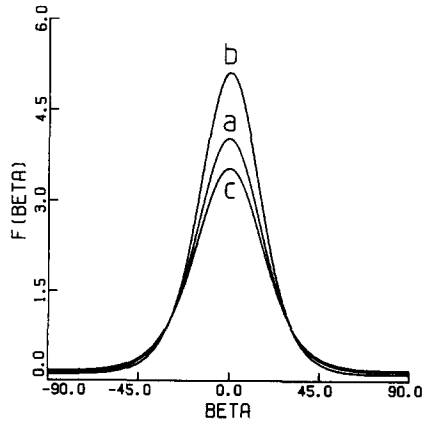

Fig. 3. Orientational distribution function $f(\beta)$ of CSL molecules embedded in multilamellar liposomes of (a) DOPC: $\langle P_2 \rangle = 0.50$, $\langle P_4 \rangle = 0.25$; (b) DOPC with 20 mol% cholesterol, 7-dehydrocholesterol, β-sitosterol or stigmasterol: $\langle P_2 \rangle = 0.58$, $\langle P_4 \rangle = 0.32$; (c) DOPC with 20 mol% estradiol: $\langle P_2 \rangle = 0.46$, $\langle P_4 \rangle = 0.22$; all at $T = 35$ °C.

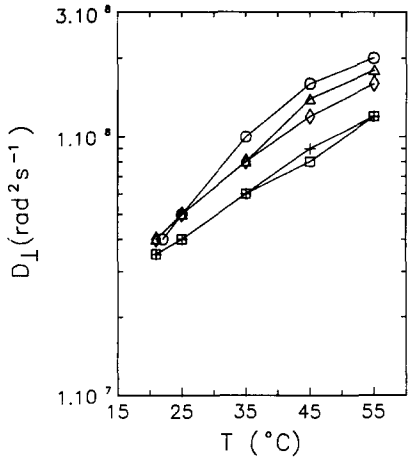

Fig. 4. Temperature dependence of the rotational diffusion coefficient $D_\perp$ of CSL molecules embedded in multilamellar liposomes of DOPC with and without 20 mol% steroids. The data for DOPC without steroids were taken from Ref. 20. (○) DOPC without steroids, (□) DOPC with cholesterol, 7-dehydrocholesterol, β-sitosterol or stigmasterol, (+) DOPC with lanosterol, (◇) DOPC with ergosterol, (△) DOPC with estradiol.

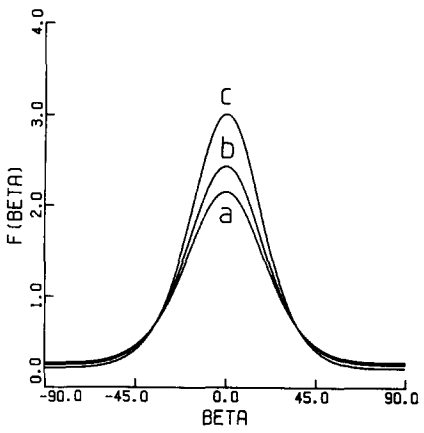

Fig. 6. Orientational distribution function $f(\beta)$ of CSL molecules embedded in multilamellar liposomes of (a) DGDG: $\langle P_2 \rangle = 0.30$, $\langle P_4 \rangle = 0.12$; (b) DGDG with 20 mol% stigmasterol: $\langle P_2 \rangle = 0.34$, $\langle P_4 \rangle = 0.14$; (c) DGDG with 20 mol% 7-dehydrocholesterol: $\langle P_2 \rangle = 0.40$, $\langle P_4 \rangle = 0.18$; all at $T = 35\,^\circ$C.

with the experimental error, only appears on raising the temperature to over 35 °C.

The addition of cholesterol, 7-dehydrocholesterol, β-sitosterol, stigmasterol and lanosterol decreases the rotational diffusion coefficient, $D_\perp$, in the temperature interval 35–55 °C. However, a significantly smaller effect is found for ergosterol and estradiol. The tendency of estradiol to decrease $D_\perp$ is surprising, as it is observed to cause a reduction in the order parameter $\langle P_2 \rangle$.

It is important to note here that in all our stimulations the motional anisotropy $N = D_\parallel / D_\perp$ was fixed at a value of 5. No significant changes in the quality of the fits were obtained on varying N between 5 and 8 and the addition of steroids appears to have no consistent effect on this parameter.

DGDG multilamellar liposomes

The order parameters $\langle P_2 \rangle$ of CSL, embedded in multilamellar liposomes of DGDG, with and without 20 mol% 7-dehydrocholesterol and stigmasterol are shown in Fig. 5.

The orientational order in pure DGDG bilayers is considerably lower than in pure DOPC bilayers (cf. Fig. 2 and Fig. 5). The orientational distribution functions for the various DGDG lipid systems, at $T = 35\,^\circ$C, are given in Fig. 6. It can be seen that the incorporation of 20 mol% 7-dehydrocholesterol causes a strong increase in $\langle P_2 \rangle$, and hence in the degree of orientational order. DGDG/stigmasterol systems also exhibit a higher degree of orientational order than the pure DGDG bilayers, but the effect is smaller than in DGDG/7-dehy-

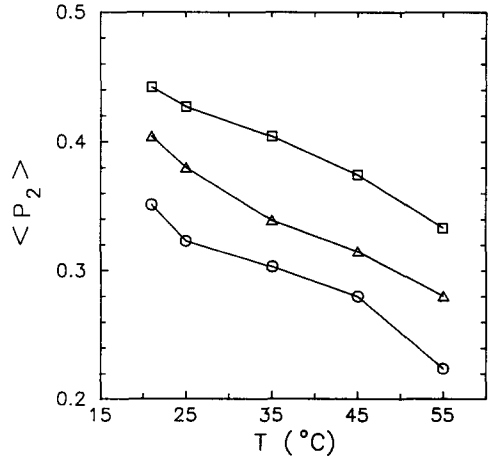

Fig. 5. Temperature dependence of the order parameter $\langle P_2 \rangle$ of CSL molecules embedded multilamellar liposomes of DGDG, (○) DGDG, (□) DGDG with 20 mol% 7-dehydrocholesterol. (△) DGDG with 20 mol% stigmasterol.

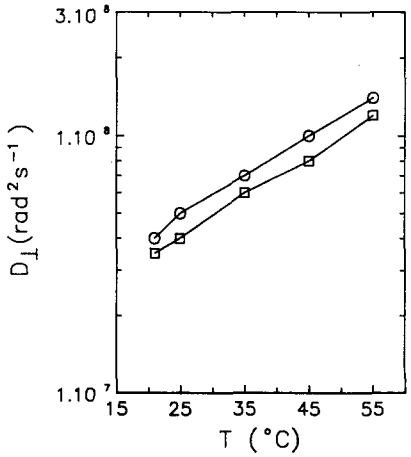

Fig. 7. Temperature dependence of the rotational diffusion coefficient $D_\perp$ of CSL molecules embedded multilamellar liposomes of DGDG. (○) DGDG with and without 20 mol% stigmasterol, (□) DGDG with 20 mol% 7-dehydrocholesterol.

drocholesterol liposomes. This contrasts with the situation in DOPC bilayers, where we found no differences between the effect of these two steroids on the molecular ordering.

The temperature dependencies of $D_\perp$ in DGDG lipid systems with and without the steroids are given in Fig. 7. In pure DGDG bilayers, $D_\perp$ is smaller than in pure DOPC bilayers (cf. Fig. 4 and Fig. 7). Interestingly, we find the same values of $D_\perp$ in both DGDG and DGDG/stigmasterol bilayers over the whole temperature range studied. Furthermore, 7-dehydrocholesterol causes only a small decrease in the rate of rotational diffusion. However, the proportional decrease of $D_\perp$ is not temperature dependent, in marked contrast to the findings for DOPC bilayers.

Again, no consistent effect of the addition of the steroids on the motional anisotropy, N, was observed.

Discussion

The effects of steroids on the orientational order and reorientational dynamics in lipid bilayers systems have been studied widely in the past using ESR techniques [12–14,16,42–47]. The ESR spectra observed from nitroxide fatty-acid or CSL probes embedded in the lipid bilayer were interpreted on the basis of the extreme motional-narrowing approximation [48]. However, we have recently shown, that while this approach yields reasonable values for the order parameter $\langle P_2 \rangle$ in lipid dispersions, it seriously overestimates the absolute rates of motional rotation rates [20]. A direct comparison between the diffusion constants reported here and the rotational correlation times, τ, widely found in the literature, is hampered by the fact that these quantities are not generally related in a transparent way [30,49]. A conversion from diffusion constants to correlation times can only be carried out through the solution of the anisotropic diffusion equation which takes into account the orienting potential, Eqn. 1, experienced by the spin-label molecules.

The results presented above show that the incorporation of sterols into lipid bilayers can induce a modulation of both the degree of orientational order and rates of rotational diffusion. These findings are in qualitative agreement with the earlier studies in which only phosphatidylcholine systems containing sterols were used.

The ESR spectra of CSL molecules in DOPC bilayers containing 20 wt% of cholesterol, 7-dehydrocholesterol, β-sitosterol, stigmasterol and lanosterol were essentially identical, indicating that these sterols induced the same effects on the lipid bilayer over the temperature range studied. The incorporation of these sterols into the DOPC bilayers enhances the orientational order of the CSL molecules at every temperature studied, but only induces a pronounced slow-down in their rotational motions at temperatures above 35 °C. Similar results were obtained in DOPC/ergosterol liposomes, but the changes are now less pronounced than in the other five DOPC/steroid systems.

In contrast, the addition of stigmasterol to DGDG bilayers appears to increase $\langle P_2 \rangle$, without affecting the diffusion coefficients. Furthermore, the incorporation of 7-dehydrocholesterol to DGDG bilayers causes a large enhancement in the orientational order, but has only a small effect on $D_\perp$ of the CSL molecules. Importantly, this latter effect appears to be independent of temperature.

The effects of estradiol on the order and dynamics of DOPC bilayers do not fit into the pattern described above. The order parameter $\langle P_2 \rangle$ is significantly lowered on the introduction of 20 wt% of the steroid, but with a concomitant decrease in the diffusion coefficients. The main structural difference between estradiol and the other steroids used in this study is that the side chain is replaced by a second hydroxyl group. The molecules thus have two hydrophylic groups at opposite sides and are expected to be embedded in the bilayer in a different way than the steroids with a hydrophobic side chain. Our data show that this results in a lower average order and slower rotational motions in the DOPC bilayer. This finding is at variance with common concepts about membrane fluidity [2,50], which correlate a lower order with an increase in the rotational mobility.

The results presented here do not support the hypothesis of a universal relation between the order parameter $\langle P_2 \rangle$ and the diffusion coefficient $D_\perp$ of CSL molecules in POPC/cholesterol bilayers put forward by Shin and Freed [18]. This relationship, $D_\perp = A(1 - \langle P_2 \rangle)^2$, has been found to hold in planar multibilayer systems. We believe that the reason for this discrepancy must be sought in our earlier observations that $\langle P_2 \rangle$ exhibits a stronger decrease with temperature in multilamellar liposomes than in planar multibilayers of POPC, while a similar dependence of $D_\perp$ on temperature is observed for both configurations [19,20].

In the numerical simulations of the observed ESR spectra we have taken the rates of rotational motions of the CSL molecules about their long axes, as reflected in the diffusion coefficient $D_\parallel$, to be totally correlated with the rates of rotation of the long axes, $D_\perp$, in the bilayer system. This correlation was introduced by keeping the motional anisotropy N equal to 5. While this was done primarily to reduce the number of free parameters entering the calculations, we have found no significant improvements in the fits on varying N in the range 5–8. However, the quality of the fits deteriorated markedly on taking values for N outside this range, particularly on increasing N beyond 10. Our findings contrast with those of Shin and Freed [18] where an increase of N with increasing cholesterol content was reported in oriented, planar POPC/cholesterol multibilayers. N was found to increase from about 5 in pure

POPC multibilayers to about 70 in multibilayers containing 30 mol% cholesterol. This observation, however, is based on spectral simulations of ESR spectra obtained at a single orientation with the sample plane perpendicular to the static magnetic field. It is remarked, however, that at this orientation the simulated lineshapes are considerably more sensitive to the value of $D_\perp$ than to that of $D_\parallel$. Indeed, we have also previously argued that a reliable value for N can only be obtained from simulations of angle-resolved ESR spectra [19]. Nevertheless, we note that a similar dependence of N on cholesterol concentration has also been reported for planar egg phosphatidylcholine multibilayers, but using a fast motional analysis [45].

The marked changes in the rates of the rotational motion brought about by the addition of steroids contrasts with the lack of a significant effect of unsaturation on the bilayer dynamics [19,20]. In this context we note that the lower values of $D_\perp$ found in the pure DGDG than in the pure DOPC liposomes, appear to reflect the lesser hydration of the former systems. It is known that the DGDG bilayers incorporate a maximum of about 22 wt% of water [51] compared to a hydration rate of 35% for DOPC [52]. The presence of the polyunsaturated fatty acid chains in DGDG is nevertheless reflected in the lower values of the order parameter $\langle P_2 \rangle$.

We have previously shown that for a variety of lipid systems an increase in the hydration of the headgroups results in a lowering of $\langle P_2 \rangle$ and an increase of the diffusion coefficient $D_\perp$ of the CSL molecules [19,20]. The question now arises as to whether the effects reported here can be ascribed to changes in the hydration state of the headgroups caused by the anchoring of steroid molecules at the aqueous interface of the bilayers. While such an effect cannot be completely ruled out, we note that the incorporation of 20 wt% of cholesterol into DOPC bilayers, has a more pronounced effect on $\langle P_2 \rangle$ and a smaller effect on $D_\perp$ than reducing the water content from 35 to 24 wt% [20]. Moreover, the differential temperature effect on the order and dynamics of DOPC systems appears to be confined to the steroid-containing liposomes.

Conclusions

The ESR spectra of CSL in DOPC bilayers containing 20 wt% of cholesterol, 7-dehydrocholesterol, β-sitosterol, stigmasterol and lanosterol were essentially identical, indicating that these steroids induced the same effects on the lipid bilayer over the temperature range studied, 21–55%. The incorporation of these steroids into the DOPC bilayers enhances the orientational order of the CSL molecules at every temperature studied, but only induces a pronounced slow-down in their rotational motions at temperatures above 35 °C. Similar results were obtained in DOPC/ergosterol multilamellar liposomes, but the changes are now less pronounced than in the other five DOPC/steroid systems.

In contrast, the addition of stigmasterol to DGDG bilayers appears to increase the order parameter $\langle P_2 \rangle$, without affecting the diffusion coefficient. Furthermore, the incorporation of 7-dehydrocholesterol to DGDG bilayers causes a large enhancement in the orientational order, but has only a small effect on $D_\perp$ of the CSL molecules. Importantly, this latter effect appears to be independent of temperature.

The marked changes in the rates of the rotational motion brought about by the addition of steroids, contrasts with the lack of a significant effect of unsaturation on the bilayer dynamics [19,20]. In this context we note that the lower values of $D_\perp$ found in the pure DGDG than in the DOPC liposomes, appear to reflect the lesser hydration of the former systems.

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