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# Influence of Cholesterol and Water Content on Phospholipid Lateral Diffusion in Bilayers<sup>†</sup>

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The dependence of lipid lateral diffusion on temperature, cholesterol, and water contents has been studied in oriented bilayers in three phosphatidylcholine lipid systems and one sphingomyelin system. The lateral diffusion of lipids is found to be reduced both by the addition of cholesterol and by decreasing the water content. This reduction can be ascribed to the increase in the ordering of the lipid acyl chains upon cholesterol addition and/or reduced water content. The dependence of the lateral diffusion coefficient on the water content is similar in both the liquid ordered and the liquid disordered phase, while the apparent activation energy for the diffusion process is larger in the liquid ordered phase. We also report an anomalous increase in the lipid diffusion upon small additions of cholesterol to bilayer systems at low water content and temperatures. The increased diffusion is tentatively explained by a reduction of lipid chain entanglements.

## Introduction

Cholesterol (CHOL), phosphatidylcholine (PC), and sphingomyelin (SM) are main components of mammalian plasma membranes. A large number of studies on the physicochemical properties of this steroid molecule have been published in the past, and recently a new interest has appeared with the discovery of the importance of CHOL for domain formation in lipid membranes, often referred to as rafts.<sup>1</sup> The mechanism behind the establishment of these domains is not yet fully understood. The first detailed phase diagram of the ternary system dipalmitoylphosphatidylcholine, CHOL, and water was determined with <sup>2</sup>H NMR spectroscopy and calorimetry.<sup>2</sup> The most striking property of this phase diagram is the existence of two lamellar liquid crystalline (*L<sub>α</sub>*) phases, the liquid disordered (*L<sub>d</sub>*), and the liquid ordered (*L<sub>o</sub>*) phases, and they were reproduced also in a theoretical phase diagram.<sup>3</sup> While both the *L<sub>d</sub>* and *L<sub>o</sub>* phases exhibit the fast rotational and translational motion characteristic for a liquid crystalline phase, the acyl chains of the *L<sub>o</sub>* phase are highly ordered.<sup>2</sup> The phase behavior of systems with other saturated phospholipids and CHOL is similar, the main difference being a translation along the temperature axis for different acyl chain lengths.<sup>4</sup> Recently, we utilized the pulsed field gradient (pfg) <sup>1</sup>H NMR method to distinguish between the *L<sub>d</sub>* and *L<sub>o</sub>* phases in lipid–cholesterol systems from an analysis of determined lipid lateral diffusion coefficients.<sup>5</sup> These studies were performed on oriented lipid bilayers close to maximum hydration. In this study some important conclusions of possible biological relevance were reached; there is a fast chemical exchange of lipids between *L<sub>o</sub>* domains floating around (rafts) in a sea of an *L<sub>d</sub>* lipid bilayer (or vice versa), and the size of the domains was estimated to be in the order of micrometers or less. Moreover, the diffusion is fast enough to allow an enzyme or protein attached to the cell membrane by, e.g., one or more hydrocarbon chains (as for the caveolins) to partition

between the two phases. This means that such proteins can be activated by incorporation into the domains of high molecular ordering and a high cholesterol content.

It has been shown that high concentrations of CHOL result in an increase in the water uptake in the dimyristoylphosphatidylcholine (DMPC)/cholesterol systems.<sup>6</sup> Moreover, in a molecular dynamic simulation it was found that inclusion of CHOL decreases interlipid links and increases hydration in the polar region of a DMPC membrane.<sup>7</sup> Recently, Sparr et al.<sup>8</sup> presented a partial phase diagram for the DMPC/CHOL/water system from an investigation by isotherm sorption microcalorimetry and <sup>2</sup>H NMR spectroscopy. To get a better understanding of the effect of CHOL and water concentration on the lipid lateral diffusion coefficient, *D<sub>L</sub>*, we have performed a pfg-NMR study on three different PCs and one SM lipid system varying the CHOL and water contents.

## Materials and Methods

The compounds used for the preparation of macroscopically aligned bilayers were 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), chicken egg yolk sphingomyelin (SM), containing mostly palmitoyl chains, and 5-cholesten-3 $\beta$ -ol (CHOL). DOPC, POPC, and DMPC were from Avanti Polar Lipids, Birmingham, AL, while SM and CHOL were from Sigma, St. Louis, MO.

Deuterated water (<sup>2</sup>H<sub>2</sub>O, 99.7%) was purchased from Larodan AB, Malmö, Sweden.

Several samples were prepared for each lipid/CHOL system with the CHOL content varied from 0 up to 48 mol %. The lateral diffusion coefficient (*D<sub>L</sub>*) was then measured as a function of both temperature and water content. Details on the preparation of the samples and the pfg-NMR measurements can be found elsewhere.<sup>5</sup>

## Results

The signal decay for the lipids in the pfg-NMR experiments in previous,<sup>5</sup> as well as in this study showed a single exponential. This indicates that the observed lipids are

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(1) Simons, K.; Ikonen, E. *Nature* **1997**, *387*, 569–572.  
(2) Vist, M. R.; Davis, J. H. *Biochemistry* **1990**, *29*, 451–464.  
(3) Ipsen, J. H.; Kalström, G.; Mouritsen, O. G.; Wennerström, H. W.; Zuckermann, M. J. *Biochim. Biophys. Acta* **1987**, *905*, 162–172.  
(4) Thewalt, J. L.; Bloom, M. *Biophys. J.* **1992**, *63* (4), 1176–1181.  
(5) Filippov, A.; Orädd, G.; Lindblom, G. *Biophys. J.* **2003**, *84*, 3079–3086.

(6) Bach, D.; Miller, I. R. *Biochim. Biophys. Acta* **1998**, *1368*, 216–224.

(7) Pasenkiewicz-Gierula, M.; Rog, T.; Kitamura, K.; Kusumi, A. *Biophys. J.* **2000**, *78*, 1376–1389.

(8) Sparr, E.; Hallin, L.; Markova, N.; Wennerström, H. *Biophys. J.* **2002**, *83*, 2015–2025.

**Table 1. Lateral Diffusion Coefficient ( $10^{-12}$  m<sup>2</sup>/s) of Phospholipids in Bilayers at 55 wt % of Water<sup>a</sup>**

phospholipid	CHOL, mol %	phase	298 K	303 K	308 K	313 K	318 K	323 K	333 K
DOPC	0	$l_d$	9.32	11.5	13.7	16	18.9	22.2	29.3
	14.5	$l_d$	7.87	9.9	12.0	14.2	16.6	19.3	25.5
			84%	86%	88%	89%	88%	87%	87%
	42	$l_d$	4.94	6.75	7.83	10.0	11.9	13.7	19.6
POPC	0	$l_d$	8.87	10.7	12.6	15.3	17.7	20.6	28.3
	13	$l_d$	7.36	9.00	10.7	12.9	15.1	17.4	24.5
			83%	84%	85%	84%	85%	84%	87%
	48	$l_d$	3.73	4.35	5.50	7.00	8.60	10.9	16.2
DMPC	0	$l_d$	5.70	9.00	11.5	14.3	18.0	22.3	30.9
	5	$l_d$	4.40	8.20	10.9	14.5	17.9	22.0	30.5
			77%	91%	95%	101%	99%	99%	99%
	33	$l_o$	1.54	2.27	3.40	4.73	6.58	8.70	15.1
SM	0	$l_d$	27%	25%	29%	33%	37%	39%	49%
	10.5	$l_d + l_o$					5.83	7.65	12.1
							4.05	6.02	9.66
	31	$l_o$				1.19	69%	79%	79%
							1.74	2.52	5.15
							30%	33%	43%

<sup>a</sup> The percentage values give the ratio of  $D_L(\text{CHOL})/D_L(0)$  for each of the specified CHOL contents.

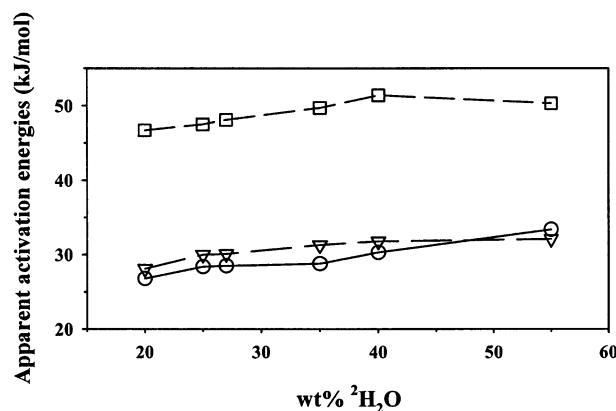
either in a single phase or in fast exchange between two or more phases. The observed narrowing of the NMR line widths when the normal of the glass plates is at the magic angle with respect to the main magnetic field also ensured that the samples remained oriented and in the  $L_\alpha$  phase for all temperatures and water contents studied.

**Lateral Diffusion Coefficients for Bilayers in Equilibrium with Excess Water.** The highest water content used in this report was 55 wt %  $^2\text{H}_2\text{O}$ . At this water content the appearance of small water droplets on the sample walls of all samples indicated that the bilayers had reached their maximum hydration. In Table 1 we report the values of  $D_L$  obtained at this water content.

**Dependence of  $D_L$  on Temperature.** The temperature dependence of  $D_L$  could be described by an equation of the Arrhenius type,  $D(T) = D_0 \exp(-E_A/RT)$ , for the DOPC and POPC samples. In bilayers containing DMPC or SM, the temperature dependence deviates from the Arrhenius type at temperatures close to the main transition temperatures (312 and 297 K, respectively).<sup>5</sup> Because of this, apparent activation energies for DMPC were estimated in the high-temperature interval, where such effects are absent. For SM the rather broad transition from the gel to liquid crystalline phase caused by chain heterogeneity further impedes such analysis. Moreover, it was shown earlier that SM samples with 10.5 mol % CHOL and 35 wt %  $^2\text{H}_2\text{O}$  were in the two-phase area of  $l_d$  and  $l_o$  phases and that the temperature dependence therefore deviated from the Arrhenius type.<sup>5</sup> No estimation of activation energies has therefore been made for the SM system.

The obtained apparent activation energies were found to be independent of the water content for the PC systems. Especially for the unsaturated lipids, the changes in  $E_A$  were on the order of the estimated accuracy of the measurement and therefore only the mean values obtained for water contents between 20 and 55 wt % are reported for three different CHOL contents (Table 2). For the DMPC system, a slight increase in  $E_A$  with increasing water content could be observed. Furthermore, there is a marked increase in the  $E_A$  values obtained at high CHOL content (Figure 1).

**Dependence of  $D_L$  on Water Content.** To facilitate a comparison of the effect of a change in the water content between the different systems, we report the normalized diffusion coefficients  $D_{\text{rel}} = D/D(55)$ , where  $D(55)$  is the value of the diffusion coefficient obtained at 55 wt % water (Table 1). Figure 2 shows representative values of  $D_{\text{rel}}$  for



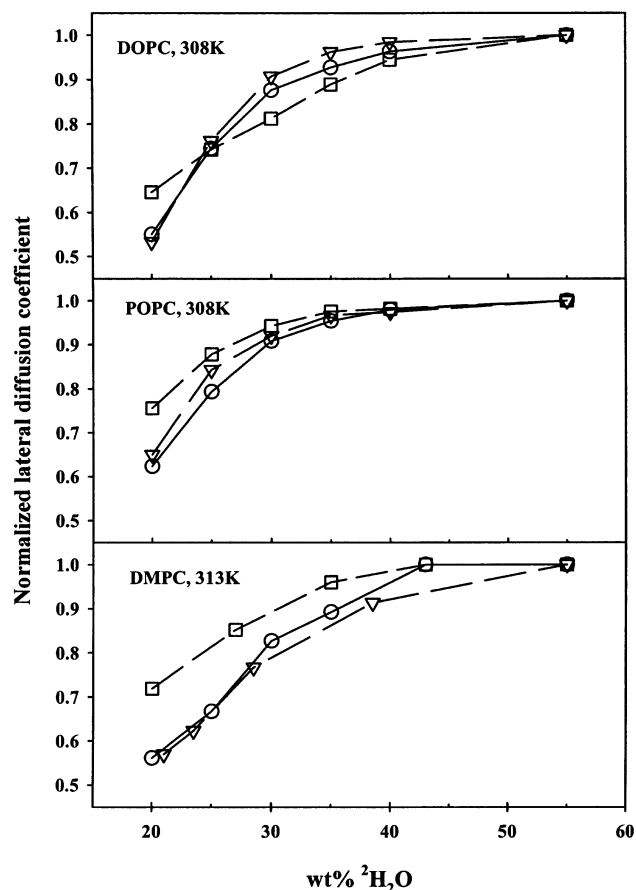
**Figure 1.** Apparent activation energies obtained in the temperature range of 298–313 K in the DMPC/CHOL system. Data are shown for 0 (circles), 5 (triangles), and 33 (squares) mol % of CHOL.

**Table 2. Mean Values of the Apparent Activation Energies,  $E_A$ , Obtained for the DOPC/CHOL and the POPC/CHOL Systems at Water Content between 20 and 55 wt % and in a Temperature Range of 303–333 K**

lipid	mol % CHOL	$E_A$ (kJ/(mol K))
DOPC	0	24
	14.5	24
	42	29
POPC	0	27
	13	27
	48	35

the PC systems. Since  $E_A$  is virtually independent of the water content for these systems, the data for different temperatures will fall on the same curve and, therefore, data for only one temperature is shown in the figure. The dependence on the water content is qualitatively the same for all three lipids and also for the different CHOL contents.  $D_{\text{rel}}$  falls rapidly in the range of 20–35 wt % water down to values of 0.5–0.7. For the DOPC and POPC systems, addition of CHOL results in a slightly smaller reduction in  $D_{\text{rel}}$  but for the DMPC system no obvious trend is seen.

The corresponding plot of  $D_{\text{rel}}$  as a function of the water content for the SM system is shown in Figure 3 for 323 K. Similar curves were obtained also at the other studied temperatures. At 0 and 11 mol % CHOL,  $D_{\text{rel}}$  is reduced to about 0.6 with the largest change in the region of 30–40 wt % water. For 31 mol % CHOL, the reduction is smaller.



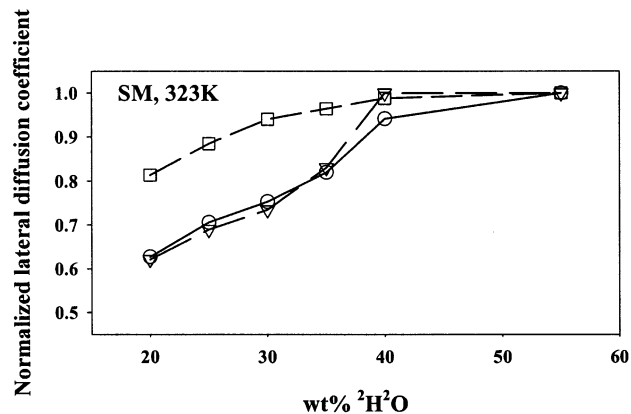
**Figure 2.** Normalized diffusion coefficients as a function of the water content at the indicated temperatures for the DOPC (top), POPC (middle), and DMPC (bottom) systems. The CHOL content is as follows: for DOPC, 0 (circles), 14.5 (triangles), and 42 (squares) mol %; for POPC, 0 (circles), 13 (triangles), and 48 (squares) mol %; and for DMPC, 0 (circles), 5 (triangles), and 33 (squares) mol %.

**Dependence of  $D_L$  on CHOL Content.** In general  $D_L$  was found to decrease monotonously with increasing CHOL content. However, in the DOPC/CHOL system at low hydration small additions of CHOL result in an increased diffusion coefficient (Figure 4). This effect gradually disappears at higher temperatures and for higher water contents.

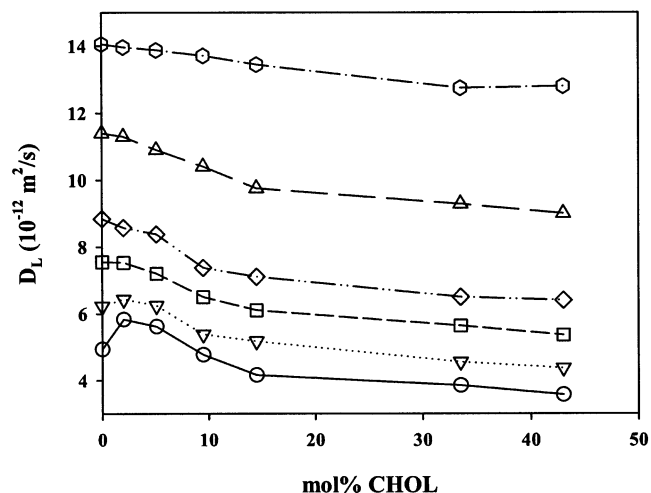
### Discussion

**Lateral Diffusion Coefficients for Bilayers in Equilibrium with Excess Water.** The  $D_L$  dependence on CHOL at excess water reported in Table 1 is essentially the same as that reported earlier for 30 and 35 wt % water,<sup>5</sup> since the diffusion coefficient is independent of the hydration at high (>35 wt %  $^2\text{H}_2\text{O}$ ) water content (Figures 2 and 3). CHOL induces a reduction of  $D_L$  of roughly 1% for each mol % of added CHOL to about 40–60% of the value for pure lipid in the DOPC and POPC systems and, in general, the reduction is slightly larger for the lower temperatures. These systems are in the  $l_d$  phase at all concentrations and temperatures, and the reduction in  $D_L$  can be explained by the ordering effect of CHOL, which is believed to reduce the free volume available for the diffusion process.<sup>9</sup>

In the DMPC system a similar effect of CHOL is observed for the 5 mol % sample, which is in the  $l_d$  phase.



**Figure 3.** Normalized diffusion coefficients at 323 K as a function of the water content for the SM system. The CHOL content is 0 (circles), 11 (triangles), and 31 (squares) mol %.



**Figure 4.** Lateral diffusion coefficients in the DOPC system at 20 wt %  $^2\text{H}_2\text{O}$  as a function of the CHOL content at temperatures: 298 (circle), 303 (triangle down), 308 (square), 313 (diamond), 323 (triangle up), and 333 K (hexagon).

For the 33 mol % sample, the reduction is larger than expected from the trend in the  $l_d$  phase. This is in agreement with the tentative phase diagram in which the 33 mol % sample resides in the  $l_o$  phase<sup>5,10</sup> and the additional reduction can be ascribed to the large ordering in lipid acyl chains characteristic for the  $l_o$  phase.

Finally, in the SM system the reduction of  $D_L$  at 31 mol % CHOL is comparable to that in the DMPC system at 31 mol % CHOL. This is in accordance with previous studies in which it was found that this sample is in the  $l_o$  phase. The sample with 10.5 mol % CHOL is in the two-phase area of  $l_d$  and  $l_o$  phases, and the reduction in  $D_L$  is therefore larger than that expected for the  $l_d$  phase.

The apparent activation energies,  $E_A$ , for the diffusion process are only slightly dependent on the CHOL content within the same phase. There is, however, large differences between the  $E_A$  values obtained in the  $l_d$  and the  $l_o$  phases. For the DOPC and the POPC systems and for 0 and 5 mol % CHOL in the DMPC system,  $E_A$  is in the range of 25–35 kJ/mol (Table 2 and Figure 1). These samples are all in the  $l_d$  phase. For the DMPC sample with 33 mol % CHOL, which is in the  $l_o$  phase,  $E_A$  is significantly higher (around 50 kJ/mol) (Figure 1). This difference in  $E_A$  between the two phases might be coupled to the different ordering of the lipid acyl chains. A square dependence of the order

(9) Polson, J. M.; Vattulainen, I.; Zhu, H.; Zuckermann, M. J. *Eur. Phys. J.* **2001**, *E5*, 485–497.

(10) Almeida, P. F. F.; Vaz, W. L. C.; Thompson, T. E. *Biochemistry* **1992**, *31*, 6739–6747.



parameter of the molecule on the  $E_A$  of the diffusion process has been suggested, both empirically<sup>11</sup> and based on the free volume theory.<sup>12</sup> Such a dependence of the order parameter on  $E_A$  might also explain the slight decrease in the  $E_A$  values observed upon gradually increasing the unsaturation of the lipids in the succession DMPC  $\rightarrow$  POPC  $\rightarrow$  DOPC (Table 2 and Figure 1). It is however unclear why the general ordering of the acyl chains upon increasing amounts of CHOL in the  $l_d$  phase has such a small effect on the activation energy.

**Dependence of  $D_L$  on Water Content.** In general, a reduction of the water content from maximally swollen bilayers will first result in a reduction of the water layer thickness, and as the water content is reduced below the complete hydration of the lipid headgroups, there will be a decrease of the lipid headgroup area and consequently an increase in the effective hydrocarbon chain length of the lipids. This effect has been observed by X-ray in a variety of bilayer systems where the repeat distances can be divided into a partial lipid thickness and a partial water thickness.<sup>13</sup> In all studied PC systems a decrease in the water content results in an increase in the partial bilayer thickness, and this change in effective hydrocarbon length must be accompanied by an increase in the chain ordering. This would in turn affect the lateral diffusion of the lipids, and  $D_L$  is expected to decrease with decreasing water content. Further reduction in  $D_L$  might appear because of obstruction effects caused by the proximity of the headgroups protruding into the water phase from opposing bilayers.<sup>14,15</sup> Taken together, a reduction of the water content is expected to produce a reduction of  $D_L$  for the lipids in the bilayers.

Figure 2 presents the normalized diffusion coefficients,  $D_{rel}$ , obtained for the three PC systems.  $D_{rel}$  shows the expected reduction from the value obtained at excess water and the reduction is fairly similar for all three systems. For small additions of CHOL, the curves drop steeply at water content between 20 and 30 wt %, corresponding to 8–15 water/DMPC and 10–17 water/DOPC or POPC. This is the range of water content in which the hydration shell of the lipid headgroups is completed and one-dimensional swelling of the bilayers begins.<sup>13,16–18</sup> The largest reduction in  $D_L$  is thus coupled to changes in the area of the lipid headgroup and effective chain length. The curves for more than 30 mol % CHOL seem to be somewhat less steep. This might be a consequence of a different water uptake by the lipids and the CHOL molecules. It is expected that CHOL will have fewer water molecules tightly bound than the lipids, so that the effective number of water molecules/lipid is larger than the average number of water/molecule in the mixed bilayers.

From earlier studies on these systems it was concluded that the bilayers are in the  $l_d$  phase at 35 wt % water for all CHOL content in the unsaturated systems. Given the rather large difference in  $D_L$  between the  $l_d$  and the  $l_o$  phases,  $D_{rel}$  is expected to drop significantly if the  $l_o$  phase were to be induced by the change in water content. The similarity between the curves for the pure lipids (which only forms the  $l_d$  phase) and the systems containing CHOL

suggests that these unsaturated systems only form the  $l_d$  phase also at low water contents.

In the DMPC system the  $l_d$  phase forms at 35 wt % water for 0 and 5 mol % CHOL while the sample with 33 mol % CHOL forms the  $l_o$  phase.<sup>5</sup> Despite the different phases involved in this system,  $D_{rel}$  follows the same dependence on the water content as for the unsaturated lipids. This finding indicates that the diffusion is similarly affected by changes in the water content in both the  $l_d$  and the  $l_o$  phases.

The curves for  $D_{rel}$  in the SM system shown in Figure 3 also show a reduction to 50–60% at 20 wt % water. However, the shape of the curves is slightly different than that for the PCs in that the steepest change occurs at 30–40 wt % of water for SM. This range corresponds to 14–22 water molecules/SM. These results indicate a similar ordering effect on SM upon decreasing the water content. This result is in contrast to X-ray studies of SM, in which no thickening of the bilayers was found.<sup>19</sup>

**Anomalous Diffusion at Low Water Content in the DOPC System.** At low water content we observed a deviation from the monotonic decrease in  $D_L$  with increasing CHOL content. This anomalous behavior is illustrated in Figure 4 for the DOPC system at 20 wt % of water. At high temperatures the dependence is close to linear, but as the temperature decreases, a plateau at low CHOL content becomes more and more pronounced. For the two lowest temperatures there is even a small increase in  $D_L$  for small additions of CHOL. As a possible explanation we suggest that there is an increased molecular mobility of the lipids in this region, caused by a decrease in the intermolecular interaction between the phospholipid molecules. This in turn is due to the presence of CHOL, which results in a decrease in the entanglement between the acyl chains that will be slightly ordered by the flat steroid molecule. Recently it was reported<sup>20</sup> that low amounts (<3 mol %) of CHOL contribute to a “softening” of the membrane and “rigidification” (better described by a higher molecular ordering) of the membranes occurs only at concentrations higher than 4 mol % CHOL. In a <sup>2</sup>H NMR relaxation study on deuterated lipids, Trouard et al.<sup>21</sup> also proposed that an increased rotational and translational molecular mobility at low CHOL content is caused by a decrease of entanglements between the chains. To summarize, concentrations of up to about 2–3 mol % of CHOL reduce the interaction characteristic for pure DOPC and this might be the reason for the observed increase in  $D_L$ . It is still unclear why this effect is most pronounced at low water content and low temperatures. Further studies have to be performed before a firm explanation can be given.

## Conclusions

This study has shown that the lateral diffusion of lipids is reduced both by addition of cholesterol and by decreasing water content. This dependence can be explained by the increase in ordering of the lipid acyl chains. The dependence of  $D_L$  on the water content is mainly the same in both the  $l_o$  and the  $l_d$  phases, but the apparent activation energy is found to be significantly larger in the  $l_o$  phase. At low water content and temperature, we also observe an increase in  $D_L$  upon small additions of CHOL, and this effect is tentatively ascribed to a decrease in the chain entanglements due to the incorporation of CHOL.

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(11) Shin, Y.-K.; Freed, J. H. *Biophys. J.* **1989**, *55*, 537–550.

(12) Diogo, A. C.; Martins, A. F. *J. Phys.* **1982**, *43*, 779–786.

(13) Cevc, G. *Phospholipids handbook*; Marcel Dekker: New York, 1993.

(14) Kuo, A.-L.; Wade, C. G. *Biochemistry* **1979**, *18*, 2300–2308.

(15) McCown, J. T.; Evans, E.; Diehl, S.; Wiles, H. C. *Biochemistry* **1981**, *20*, 3134–3138.

(16) Faure, C.; Bonakdar, L.; Dufourc, E. J. *FEBS Lett.* **1997**, *405*, 263–266.

(17) Hristova, K.; White, S. H. *Biophys. J.* **1998**, *74*, 2419–2433.

(18) Janiak, M. J.; Small, D. M.; Shipley, G. G. *J. Biol. Chem.* **1979**, *254*, 6068–6078.

(19) Maulik, P. R.; Shipley, G. G. *Biochemistry* **1996**, *35*, 8025–8034.

(20) Lemmich, J.; Mortensen, K.; Ipsen, J. H.; Honger, T.; Bauer, R.; Mouritsen, O. G. *Eur. Biophys. J.* **1997**, *25*, 293–304.

(21) Trouard, T. P.; Nevzorov, A. A.; Alam, T. M.; Job, C.; Zajicek, J.; Brown, M. F. *J. Chem. Phys.* **1999**, *110*, 8802–8818.