Slow motions in oriented phospholipid bilayers and effects of cholesterol or gramicidin

A ^{19}F-NMR $T_{1\rho}$ study

Zheng-yu Peng,*‡ Nico Tjandra,*‡ Virgil Simplaceanu,* and Chien Ho*
*Department of Biological Sciences and ‡Department of Physics, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213

ABSTRACT In an extension of our earlier work (Peng, Z.-y., V. Simplaceanu, I. J. Lowe, and C. Ho. 1988. *Biophys. J.* 54:81–95), the rotating-frame nuclear spin-lattice relaxation ($T_{1\rho}$) technique has been used to investigate the slow molecular motions (10^{-4}–10^{-6} s) in lipid bilayers prepared from protonated or perdeuterated ^{19}F-labeled phospholipids in the absence and presence of cholesterol or gramicidin as membrane-interacting molecules. Complications caused by the ^{19}F-^{1}H cross-polarization observed previously can be removed by the substitution of ^{2}H for ^{1}H in the acyl chains. Only a weak dependence of the $T_{1\rho}^{-1}$ on the locking field strength is found for a phospholipid molecule with perdeuterated acyl chains, indicating that there are no slow motions with a single, well-defined correlation time between 5×10^{-6} and 4×10^{-5} s. However, the orientation dependences of the $T_{1\rho}^{-1}$ can be well fitted by motional models with either one slow motion having an unspecified geometry or with a superposition of two specific types of slow motions. Cholesterol and gramicidin show distinct effects in altering either the geometry or the weighting of slow motions in phospholipid bilayers, as reflected by changes in the orientation dependence. These two additives also exhibit quite different label-position specificities. A qualitative understanding of the induced effects of cholesterol and gramicidin on the dynamics of phospholipid bilayers will be discussed.

INTRODUCTION

The phospholipid bilayer is the basic structural component of cell membranes. Under physiological conditions, the bilayer can be considered as a two-dimensional fluid, whereas in the third dimension, the lipid molecules are partially ordered. The commonly accepted model of biological membranes is the fluid mosaic model (Singer and Nicolson, 1972). In a recent publication (Peng et al., 1988), we have reported an investigation of the slow molecule motions (10^{-4}–10^{-6} s) of the phospholipid acyl chain for lipid bilayers in the liquid-crystalline phase, using the nuclear spin-lattice relaxation in the rotating-frame ($T_{1\rho}$) of ^{19}F-labeled dimyristoylphosphatidylcholines (^{19}F-DMPCs). We have now extended our investigation to observe the changes on the slow motions of phospholipids that can be induced by adding cholesterol or gramicidin to the bilayer. In addition, a perdeuterated phospholipid specifically labeled with a CF_2 group in position 7 of the *sn*-2 acyl chain, 1-(myristoyl-d_{27})-2-(7,7-difluoromyristoyl-d_{25})-*sn*-glycerol-3-phosphocholine (2-[7,7-$^{19}F_2$]DMPC-d_{52}), has been synthesized. Because the dipole moment of ^{2}H is about seven times smaller than that of ^{1}H, it is possible to reduce most of the heteronuclear dipolar interaction by substitution of ^{2}H for ^{1}H in both acyl chains, thereby eliminating the ^{19}F-^{1}H cross-polarization seen at low locking fields. Hence, this allows us to determine both the locking field and the orientation dependences of the motionally induced $T_{1\rho}^{-1}$ unambiguously.

Two highly hydrophobic molecules, cholesterol and gramicidin, have been chosen as prototypes to investigate the effects of additives on the dynamics of phospholipid bilayers. Cholesterol is commonly found in many natural membranes in ratios that range from 10 to 40 mol% (Huang, 1977; Yeagle, 1985). The presence of cholesterol in a lipid bilayer can increase the order of the phospholipid acyl chains and decrease the membrane permeability (Yeagle, 1985). Gramicidin is a pentadecapeptide produced by *Bacillus brevis*. It folds into a helical conformation and forms channels in a membrane (Andersen, 1984). Gramicidin is often considered to be a model for a membrane-intercalating peptide or protein (Cornell, 1987).

Several nuclear magnetic resonance (NMR) investigations on both static and dynamic properties of the phospholipid bilayer containing cholesterol or gramicidin have been carried out (for recent reviews, see Smith and Oldfield, 1984; Bloom and Smith, 1985). Earlier studies have shown that cholesterol increases the order parameter measured by deuterium NMR in the liquid-crystalline phase, while at low temperatures it prevents the acyl

Address correspondence to Dr. Chien Ho, Department of Biological Sciences, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213.

Dr. Peng's present address is Department of Chemistry, University of California, Berkeley, CA 94720.

0006-3495/89/11/877/09 $2.00

chains from forming a highly ordered gel phase (Jacob and Oldfield, 1979; Cornell and Keniry, 1983; Dufourc et al., 1984). On the other hand, gramicidin and several other peptides or proteins have been found to cause little effect on the chain ordering in a phospholipid bilayer (Cornell and Keniry, 1983; Davis et al., 1983; Tamm and Seelig, 1983; Chupin et al., 1987). Both additives have only a small effect on the fast motion of acyl chains as monitored by the spin-lattice relaxation rate in the laboratory frame (T_1^{-1}) (Brown et al., 1982; Cornell et al., 1982; Tamm and Seelig, 1983; Chupin et al., 1987; unpublished results of Simplaceanu, V., S. R. Dowd, Z.-y. Peng, and C. Ho).

Cornell and co-workers first determined the ratios of $T_{1\rho}^{-1}$ to T_1^{-1} for dispersions of pure phospholipids and phospholipid mixed with cholesterol or gramicidin, as well as for red blood cell membranes (Cornell et al., 1982; Cornell et al., 1983). They found that the additives have a greater enhancing effect on the low-frequency relaxation than on the high-frequency relaxation, i.e., increasing the $T_{1\rho}^{-1}/T_1^{-1}$ ratio. Because a similar high ratio of the relaxation rates was observed for the natural membranes, they concluded that biological membranes are rich in slow motions and those motions or fluctuations can be enhanced by adding foreign molecules to the pure phospholipid bilayers.

We have studied the dependence of ^{19}F $T_{1\rho}^{-1}$ on both the locking field strength and the orientation of the bilayer in the magnetic field using oriented bilayers prepared from 2-[7,7-$^{19}F_2$]DMPC-d_{52}, with or without cholesterol or gramicidin. Protonated ^{19}F-DMPCs with a CF_2 label in positions 4, 8, or 12 of the *sn*-2 acyl chain have also been used to study the effect of the additives as a function of label position. The results show that both cholesterol and gramicidin can enhance $T_{1\rho}^{-1}$ significantly, but the details of their interactions with phospholipids are quite different.

MATERIALS AND METHODS

Materials

The ^{19}F-labeled phospholipids, 2-[4,4-$^{19}F_2$]DMPC, 2-[8,8-$^{19}F_2$]DMPC, and 2-[12,12-$^{19}F_2$]DMPC, were synthesized as described previously (Engelsberg et al., 1982). The synthesis of 2-[7,7-$^{19}F_2$]DMPC-d_{52} will be described in detail elsewhere. Cholesterol and gramicidin were purchased from Sigma Chemical Co. (St Louis, MO) and were used without further purification. All other chemicals and solvents were reagent grade.

Sample preparation

The phospholipid and the additive were first codissolved in chloroform/methanol mixture (3:1, vol/vol). The solution was directly deposited onto microscope cover slips, followed by evaporation in vacuum and hydration as described previously (Peng et al., 1988).

The concentration of the additives present in the lipid samples was 10–14 wt%. The corresponding molar ratios were 1:4 to 1:5 for cholesterol and 1:20 to 1:24 for gramicidin. The choice was made to reflect approximately the natural abundance of cholesterol and membrane-bound proteins or peptides found in biomembranes.

NMR measurements

The rotating-frame spin-lattice relaxation rate was obtained by measuring the decay of the transversal component of the magnetization vector in the presence of a radio frequency field (locking field) of adjustable amplitude, rotating at the same frequency as the nuclei being observed. The experimental details are described in our previous paper (Peng et al., 1988). Care was taken to maximize the signal-to-noise ratio and to avoid sample heating. A normal ^{19}F-NMR spectrum was taken for each sample before and after the relaxation time measurement to verify that the phospholipid molecules have reached and maintained their orientation.

All NMR experiments were carried out at 32°C where bilayers are in their fluid phases. For binary mixtures consisting of DMPC and 10–20 mol% cholesterol, a fluid–fluid phase separation has been reported (Shimshick and McConnell, 1973). At the concentration of cholesterol used in our experiments, the bilayer should be predominantly in the cholesterol-rich phase. Our results would not be affected significantly even if a trace of the cholesterol-poor phase was present because the time scale for $T_{1\rho}$ is much longer than the exchange lifetime of a molecule between domains. Thus, $T_{1\rho}$ measurements reflect only the averaged properties of the phospholipid molecule in both phases. For a more detailed discussion, see Peng et al. (1989).

RESULTS

Pure-2-[7,7-$^{19}F_2$]DMPC-d_{52} bilayers

Fig. 1 illustrates the differences in the 282.4 MHz ^{19}F-NMR spectra for oriented bilayers in protonated and perdeuterated ^{19}F-labeled DMPCs. Fig. 1 *A* shows the ^{19}F-NMR spectra of oriented bilayers obtained from 2-[8,8-$^{19}F_2$]DMPC as a function of the angle (β) between the bilayer director and the static magnetic field (this figure is taken from Fig. 2 *A* of Peng et al., 1988). The linewidth of these unresolved doublets arises from ^{19}F-^{19}F and ^{19}F-^{1}H dipolar interactions which scale as the second-order Legendre polynomial $P_2(\cos\beta)$, and the center of each doublet is determined by the ^{19}F chemical shift anisotropy (CSA) of the CF_2 group. Fig. 1 *B* shows the ^{19}F-NMR spectra for oriented bilayers made of 2-[7,7-$^{19}F_2$]DMPC-d_{52}. The heteronuclear dipolar broadening is now much smaller and, at each orientation, the spectrum consists of two well-resolved peaks which result from the ^{19}F-^{19}F homonuclear dipolar interaction, whereas the center of the pair shifts as the orientation of the bilayer changes, due to the CSA of the CF_2 group.

^{19}F $T_{1\rho}^{-1}$ relaxation rates have been measured for oriented bilayers of 2-[7,7-$^{19}F_2$]DMPC-d_{52} as a function of the locking field strength and sample orientation. As

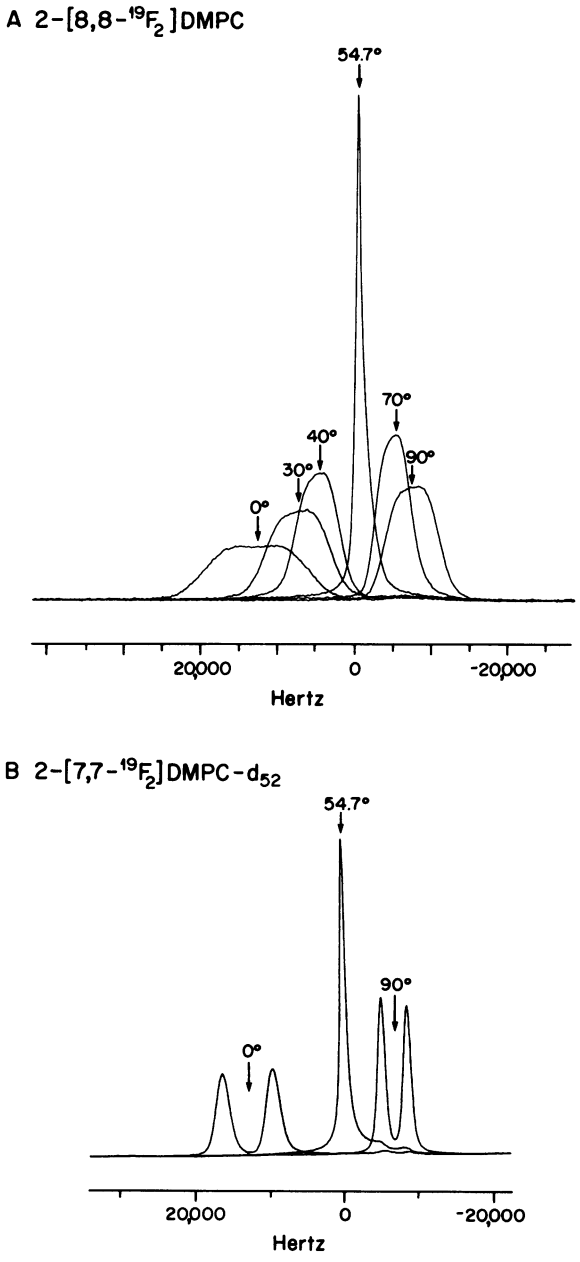

FIGURE 1 282.4-MHz ^{19}F-NMR spectra of oriented bilayers as a function of the orientation with respect to the static magnetic field: (*A*) protonated acyl chains of 2-[8,8-$^{19}F_2$]DMPC; and (*B*) perdeuterated acyl chains of 2-[7,7-$^{19}F_2$]DMPC-d_{52}. Arrows indicate the transmitter frequency for the spin-locking pulse.

shown in Fig. 2 *A*, the locking field dependence of the relaxation rate is weak for all three orientations (0, 54.7, 90°) that we have examined. The field dependence for the perdeuterated 2-[7,7-$^{19}F_2$]DMPC-d_{52} is approximately the same as previously observed for the protonated lipids only at the magic angle orientation (Peng et al., 1988).

The orientation dependence of the ^{19}F $T_{1\rho}^{-1}$ for oriented 2-[7,7-$^{19}F_2$]DMPC-d_{52} bilayers is displayed in Fig. 3 *A*, where the relaxation rate is plotted against the orientation angle β. The shape of the orientation dependence is different from that obtained for the protonated sample (Peng et al., 1988). The results obtained at 2- and 4-Gauss locking fields are quite similar, with a slightly larger difference around the 45° orientation.

Bilayers of 2-[7,7-$^{19}F_2$]DMPC-d_{52} with cholesterol or gramicidin

In agreement with previous ^{1}H-NMR investigations made on multilamellar dispersions (Cornell et al., 1982; Cornell et al., 1983), a large increase in the $T_{1\rho}^{-1}$ of the ^{19}F nuclei located in the phospholipid acyl chain has been

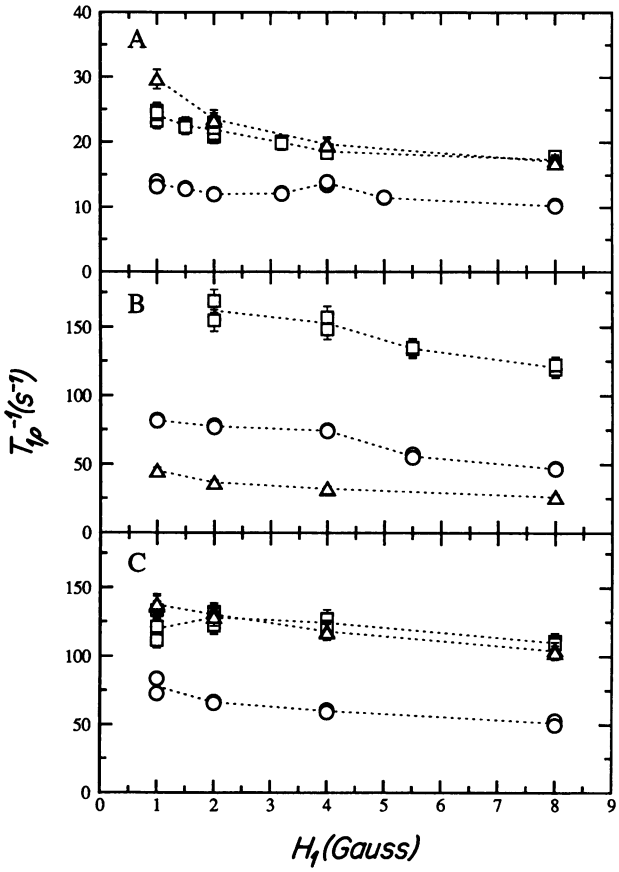

FIGURE 2 The locking field dependence of ^{19}F $T_{1\rho}^{-1}$ for oriented bilayers: (*A*) pure 2-[7,7-$^{19}F_2$]DMPC-d_{52}; (*B*) 2-[7,7-$^{19}F_2$]DMPC-d_{52} with 12 wt% cholesterol; and (*C*) 2-[7,7-$^{19}F_2$]DMPC-d_{52} with 14 wt% gramicidin. (□) 0° orientation; (△) 54.7° orientation; (○) 90° orientation. Note that *B* and *C* have different vertical scales than *A*.

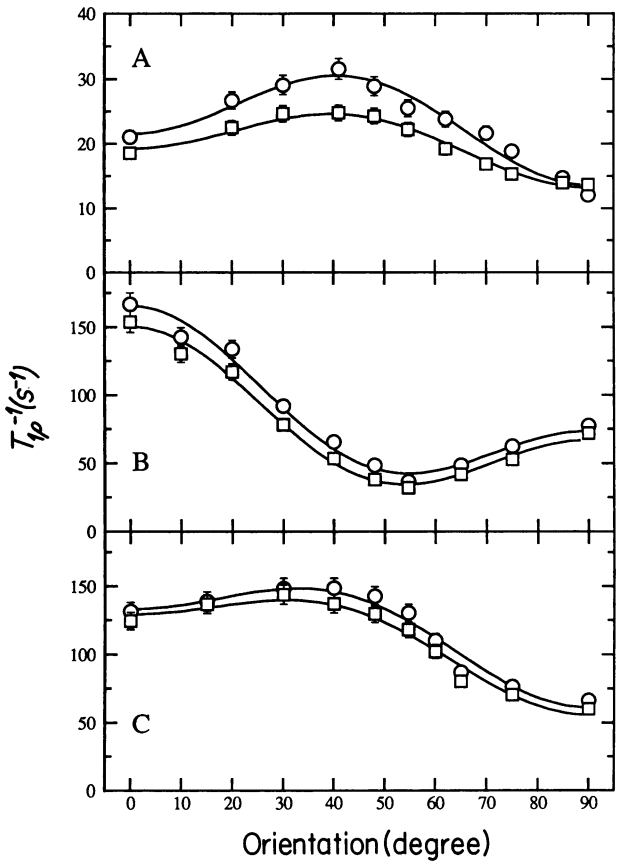

FIGURE 3 The orientation dependence of ^{19}F $T_{1\rho}^{-1}$ for oriented bilayers: (*A*) pure 2-[7,7-$^{19}F_2$]DMPC-d_{52}; (*B*) 2-[7,7-$^{19}F_2$]DMPC-d_{52} with 12 wt% cholesterol; and (*C*) 2-[7,7-$^{19}F_2$]DMPC-d_{52} with 14 wt% gramicidin. (○) H_1 = 2 Gauss; (□) H_1 = 4 Gauss. Solid lines represent the best theoretical fits using the equations from Table 2. Note that *B* and *C* have a different vertical scale than *A*.

observed after adding 12–14 wt% cholesterol or gramicidin to the oriented bilayers. In contrast, the presence of cholesterol or gramicidin causes only a small decrease in T_1^{-1}. A comparison is given in Table 1.

The locking field dependence in the presence of 12 wt% of cholesterol and 14 wt% of gramidicin, shown in Fig. 2, *B* and *C*, remains weak for all orientations and exhibits only small effects due to the presence of cholesterol or gramicidin. There is an apparent trend that both additives make the field dependence even weaker, as it can be seen from Fig. 2 for data obtained at 0 and 54.7° orientations.

Most interestingly, for the orientation dependence of $T_{1\rho}^{-1}$, the effect of adding cholesterol to the bilayer is very different from that of adding gramicidin. The results are shown in Fig. 3, *B* and *C*. The $T_{1\rho}^{-1}$ measured for 2-[7,7-$^{19}F_2$]DMPC-d_{52} bilayers containing cholesterol shows a stronger orientation dependence with an inverted shape compared to that for pure 2-[7,7-$^{19}F_2$]DMPC-d_{52} bilayers. The gramicidin causes a less drastic change in the orientation dependence, but the shape is not the same as without the additive.

TABLE 1 Spin-lattice relaxation rates measured in the rotating frame (2-Gauss locking field) and in the laboratory frame (7 × 10^4 Gauss) for oriented 2-[7,7-$^{19}F_2$]DMPC-d_{52} bilayers with and without additives

Sample	Orientation	$T_{1\rho}^{-1}$	T_1^{-1}	$T_{1\rho}^{-1}/T_1^{-1}$
	degrees	s^{-1}	s^{-1}	s^{-1}
2-[7,7-$^{19}F_2$]DMPC-d_{52}	0	21	2.3	9.4
	54.7	23	1.7	13
	90	12	1.7	7.0
+12 wt% cholesterol	0	1.6×10^2	1.6	1.0×10^2
	54.7	37	1.1	34
	90	77	1.2	63
+14 wt% gramicidin	0	1.2×10^2	2.2	56
	54.7	1.3×10^2	1.5	86
	90	67	1.4	46

Bilayers of protonated ^{19}F-DMPC with cholesterol or gramicidin

Because the protonated ^{19}F-labeled phospholipids are available for three different label positions, it can be shown that cholesterol and gramicidin exhibit label position specificity in terms of their effects on the slow motions. In Fig. 4, one can see that gramicidin enhances the relaxation of the CF_2 group in the following order: the 2-[4,4-$^{19}F_2$]DMPC bilayers are affected the most; the effect on the 2-[8,8-$^{19}F_2$]DMPC bilayers is somewhat larger than that on the 2-[12,12-$^{19}F_2$]DMPC bilayers, although both effects are relatively small. The same figure shows that cholesterol enhances the relaxation for bilayers made of any of the three lipids almost equally, with the 2-[8,8-$^{19}F_2$]DMPC, which has its label near the middle of the acyl chain, seeming to be slightly more sensitive. (Similar label-position specificity can also be found using multilamellar dispersions; see Peng [1988].)

Fig. 5 shows the orientation dependence of ^{19}F $T_{1\rho}^{-1}$ measured for bilayers of all three protonated ^{19}F-DMPCs in the presence of either cholesterol or gramicidin (10 wt%). Here, the effect of cholesterol is less visible because the orientation dependence for the pure protonated ^{19}F-DMPC bilayers (Peng et al., 1988) is different from that for the perdeuterated 2-[7,7-$^{19}F_2$]DMPC-d_{52} bilayers. However, it can be seen that the addition of gramicidin has a significant effect on the orientation dependence for 2-[4,4-$^{19}F_2$]DMPC bilayers, and the direction of the change is opposite to the change caused by the addition of cholesterol.

Both Figs. 4 and 5 show that gramicidin has its largest enhancing effect on the relaxation around the 54.7°

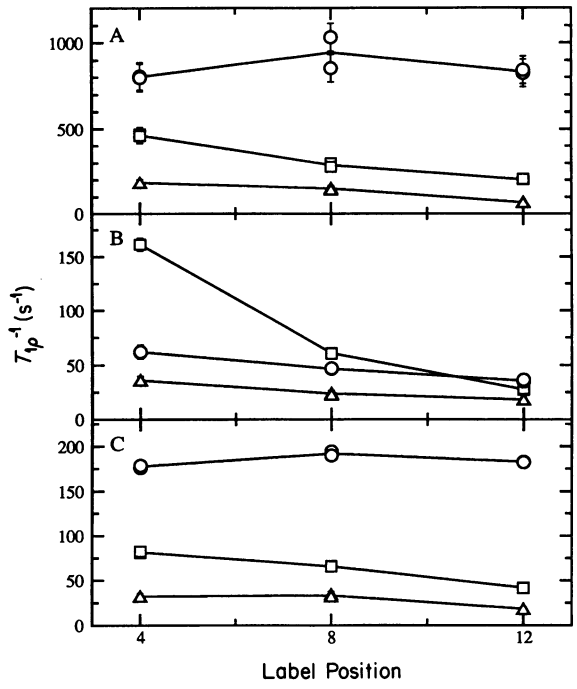

FIGURE 4 The effects of cholesterol and gramicidin on ^{19}F $T_{1\rho}^{-1}$ of oriented bilayers prepared from three protonated ^{19}F-DMPCs, plotted as a function of the label position: (*A*) 0°; (*B*) 54.7°; and (*C*) 90°. (△) pure DMPC; (○) in the presence of 10 wt% cholesterol; and (□) in the presence of 10 wt% gramicidin. Note the different vertical scales.

(magic angle) orientation, and has a relatively small effect for the 0 and 90° orientations (for the protonated ^{19}F-DMPCs). This is again in contrast to cholesterol, which shows large enhancing effects at both the 0 and 90° orientations, and only a minimal effect at the magic-angle orientation.

DISCUSSION

Differences between protonated and perdeuterated lipids

Large differences in relaxation rates between the perdeuterated and the protonated, fluorine-labeled phospholipids have been found in the 1-3 Gauss locking field range (cf. Peng et al., 1988). This indicates that the apparent $T_{1\rho}^{-1}$ for the protonated ^{19}F-DMPCs at low locking fields is dominated by an efficient cross-polarization between ^{19}F and ^{1}H (induced by mutual spin flips, or flip-flop) such that the motionally induced effects cannot be easily separated. The $T_{1\rho}^{-1}$ for 2-[7,7-$^{19}F_2$]DMPC-d_{52} at high locking fields, where cross-polarization does not pose a problem, is still smaller than the corresponding value for the protonated lipid by ~30%. This is so because the substitution of ^{1}H by ^{2}H also eliminates the heteronuclear dipolar relaxation. Approximately the same relative difference has been observed for the high-frequency, laboratory frame T_1^{-1} rates. In principle, relaxation data obtained from the perdeuterated ^{19}F-labeled phospholipid can be more easily interpreted theoretically, because the ^{19}F-^{19}F homonuclear dipolar relaxation and the CSA relaxation depend only on the orientation of the CF_2-bearing segment. In contrast, the ^{19}F-^{1}H heteronuclear dipolar contribution to relaxation depends both on the relative orientation and the distance between ^{19}F-^{1}H pairs on different carbons, and thus depends on the conformation and motion of the entire acyl chain, not to mention the second acyl chain and intermolecular interactions.

There is a discrepancy between the orientation dependence of the ^{19}F $T_{1\rho}^{-1}$ obtained for oriented 2-[7,7-$^{19}F_2$]DMPC-d_{52} bilayers (Fig. 3 *A*) and that obtained for oriented bilayers made of protonated ^{19}F-DMPCs (Peng et al., 1988). This may be explained by the following arguments: First, the orientation dependence of the protonated lipids contains a contribution from the ^{19}F-^{1}H cross-polarization which should have an orientation dependence proprotional to $P_2^2(\cos\beta)$. Although we have made an attempt to correct for this effect in the fitting, one still does not know the true relaxation rate in the absence of the ^{19}F-^{1}H spin flip-flop process. Second, as stated above, for the protonated lipids there is a heteronuclear dipolar relaxation term which depends on the motions of the entire molecule. It is quite likely that this term exhibits a different orientation dependence compared with the relaxation caused by the local modulation of the orientation of a particular CF_2 group. The second reason can also be used to explain the lack of agreement between the orientation dependence of oriented 2-[7,7-$^{19}F_2$]DMPC-d_{52} bilayers and the orientation dependence of ^{1}H $T_{1\rho}^{-1}$ reported by Pope et al. (1982). In their case, the relaxation originates from the dipolar interactions among all the protons in the molecule. Thus, the two measurements are likely to sense different motional properties.

Slow motions in pure oriented phospholipid bilayer

The use of the perdeuterated 2-[7,7-$^{19}F_2$]DMPC-d_{52} opens new possibilities to investigate the site-specific dynamics in a phospholipid bilayer. In the absence of the ^{19}F-^{1}H cross-polarization, all changes in the relaxation rate must be completely determined by changes in the molecular motions of the phospholipid. Several characteristics of slow motions in oriented phospholipid bilayers are revealed by our $T_{1\rho}$ experiments.

The weak locking field dependence shows that there are no slow motions with a single, well-defined correlation time between 5×10^{-6} and 4×10^{-5} s, which is the

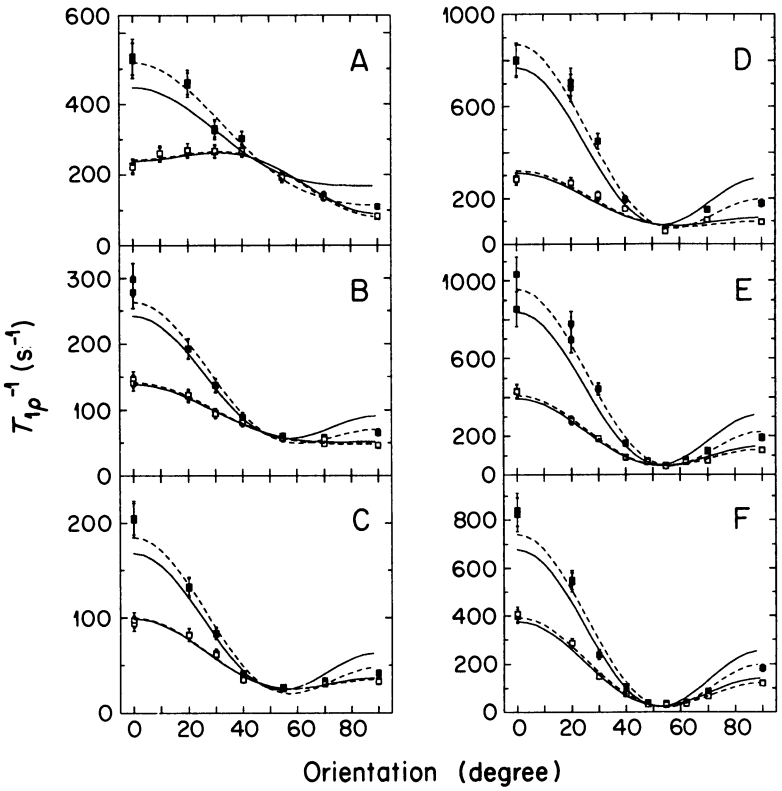

FIGURE 5 The orientation dependence of ^{19}F $T_{1\rho}^{-1}$ for oriented bilayers: (*A*) 2-[4,4-$^{19}F_2$]DMPC with 10 wt% gramicidin; (*B*) 2-[8,8-$^{19}F_2$]DMPC with 10 wt% gramicidin; (*C*) 2-[12,12-$^{19}F_2$]DMPC with 10 wt% gramicidin; (*D*) 2-[4,4-$^{19}F_2$]DMPC with 10 wt% cholesterol; (*E*) 2-[8,8-$^{19}F_2$]DMPC with 10 wt% cholesterol; and (*F*) 2-[12,12-$^{19}F_2$]DMPC with 10 wt% cholesterol. Solid lines are the best fits using the equations given in Table 2, whereas dashed lines represent fits obtained when including a ^{19}F-^{1}H cross-polarization term. (■) H_1 = 2 Gauss; (□) H_1 = 4 Gauss. Note that the vertical scale in each panel may be different.

timescale covered by our locking field range. Two possibilities should be considered. First, collective slow motions with distributed correlation times may exist on this timescale, causing the relaxation time to become slightly longer upon increasing the locking field. For example, the observed field dependence is consistent with the prediction of a director fluctuation model, although this model alone cannot explain the orientation dependence satisfactorily. Second, slow motions may be present with correlation times somewhat shorter than the timescale defined by our locking field. These motions should also produce a weak field dependence, even though their effect on the orientation dependence would be cumulative (Peng, 1988).

The orientation dependence of the pure 2-[7,7-$^{19}F_2$]DMPC-d_{52} bilayers coincides neither with the prediction of the simple, anisotropic reorientation model with $\langle P_2 \rangle = \langle P_4 \rangle$, nor with the prediction of the director fluctuation model with a small angular modulation (Peng et al., 1988, cf. Fig. 8). In the models presented above, $\langle P_2 \rangle$ and $\langle P_4 \rangle$ are time averages of the second and fourth order Legendre polynomials P_2 (cos β) and P_4 (cos β) and contain information about the geometry of the motion. Nevertheless, the orientation dependence can be fitted well by either a general anisotropic reorientation with an unspecified geometry or by our working model consisting of an anisotropic reorientation with $\langle P_2 \rangle = \langle P_4 \rangle$ plus a director fluctuation with small angular modulation (Peng et al., 1988, cf. Eqs. 20 and 25). The χ^2 of the fit for the perdeuterated lipids is much smaller than that for the protonated lipids.

The results of the curve fitting are summarized in Table 2. We have found that two interpretations for the observed orientation dependence are possible. First, the dependence could come from a single motion. If this is the case, the geometry of this motion must be quite different from either a pure anisotropic reorientation with $\langle P_2 \rangle = \langle P_4 \rangle$ or a pure director fluctuation, so that the resultant $\langle P_2 \rangle$ and $\langle P_4 \rangle$ will give the proper orientation dependence. Second, the orientation dependence could have resulted from two or more independent motions. In our working model, we have considered an example where a noncollective anisotropic reorientation with $\langle P_2 \rangle = \langle P_4 \rangle$ (possibly caused by diffusion around packing defects)

TABLE 2 Parameters obtained from fitting the orientation dependence of $T_{1\rho}^{-1}$ for oriented 2-[7,7-$^{19}F_2$]-DMPC-d_{52} bilayers with and without additives, by using the two models considered by Peng et al. (1988)

Fitted by $R[\frac{1}{5}(1 - \langle P_4\rangle) + \frac{2}{7}P_2(\cos\beta)(\langle P_2\rangle - \langle P_4\rangle) + P_2^2(\cos\beta)(\langle P_4\rangle - \langle P_2\rangle^2)]$

Sample	Locking field	$\langle P_2\rangle$	$\langle P_4\rangle$	R
	G			
2-[7,7-$^{19}F_2$]DMPC-d_{52}	2	0.44	0.04	1.4×10^2
	4	0.34	−0.01	1.1×10^2
+12 wt% cholesterol	2	0.57	0.57	5.0×10^2
	4	0.55	0.59	4.2×10^2
+14 wt% gramicidin	2	0.71	0.44	1.1×10^3
	4	0.79	0.57	1.3×10^3

Fitted by $R_1(1 - S)[\frac{1}{5} + SP_2^2(\cos\beta)] + R_2[\frac{3}{2}\sin^2\beta\cos^2\beta]$

Sample	Locking field	R_1	R_2	$S = \langle P_2\rangle - \langle P_4\rangle$
	G			
2-[7,7-$^{19}F_2$]DMPC-d_{52}	2	68	49	0.19
	4	66	34	0.14
+12 wt% cholesterol	2	5.0×10^2	≈0	0.58
	4	5.1×10^2	≈0	0.66
+14 wt% gramicidin	2	3.8×10^2	2.6×10^2	0.51
	4	4.2×10^2	2.5×10^2	0.62

together with a collective director fluctuation give the observed orientation dependence. Because the locking field dependence is weak, there is no easy way to distinguish between different motions without measuring the orientation dependence over a much broader range of locking field intensities. In practice, the lowest locking field is limited by the local field, and sample heating prevents us from reaching a much stronger locking field.

Effects of cholesterol and gramicidin

The dynamic aspects of lipid-sterol and lipid-peptide interactions are still poorly understood. In our experiments, we have found that the additives enhance the low-frequency relaxation rates ($T_{1\rho}^{-1}$), while slightly reducing the corresponding high-frequency relaxation rates (T_1^{-1}). The effect cannot be explained by considering only the fast segmental motions of the acyl chains. Instead, an enhancement of the slow motions in the phospholipid bilayer must be involved. Several mechanisms can be imagined, such as (*a*) the additive introducing new slow motions into the bilayer, (*b*) the additive enhancing the amplitude of the existing slow motions, or (*c*) the additive causing a shift of the correlation time of the existing motions. The actual effect may come from a combination of more than one mechanism. At the same percentage concentration, cholesterol usually causes a larger effect than gramicidin. The details depend on the bilayer orientation and label position. As a first step toward understanding the dynamic perturbation caused by cholesterol or gramicidin at the molecular level, we will analyze the specific information that can be extracted from our studies of the dependence of $T_{1\rho}^{-1}$ on the locking field, sample orientation, and label position.

Adding cholesterol or gramicidin to the perdeuterated 2-[7,7-$^{19}F_2$]DMPC-d_{52} bilayers has only a small effect on the locking field dependence of $T_{1\rho}^{-1}$, indicating that the distribution of correlation times around 10^{-5} s is not very sensitive to the perturbation, except that there might be a slight broadening in the distribution of correlation times. The fact that a weak field dependence is preserved implies that most of the enhancement in $T_{1\rho}^{-1}$ should come from changes in the amplitude of the existing slow motions.

Cholesterol and gramicidin exhibit distinct effects on the orientation dependence of $T_{1\rho}^{-1}$. For bilayers of 2-[7,7-$^{19}F_2$]DMPC-d_{52} containing cholesterol or gramicidin, the orientation dependences obtained can still be fitted using either the general formula for a single motion or the superposition of an anisotropic reorientation term and a director fluctuation term. The best fits are included as solid lines in Fig. 3, *B* and *C*, with the parameters presented in Table 2. It can be seen that the orientation dependence of bilayers with cholesterol can be described quite well by an anisotropic reorientation alone with $\langle P_2\rangle = \langle P_4\rangle \approx 0.6$. The effect of gramicidin is less

evident, mainly because the label at position 7 is not particularly sensitive to the presence of gramicidin.

The fitting for the orientation dependence for protonated ^{19}F-DMPC bilayers is not as good as in the case of the perdeuterated phospholipid. Similar to what has been found previously (Peng et al., 1988), the quality of the fit can be improved by including a term that accounts for the effect of the ^{19}F-^{1}H cross-polarization. In this case, cholesterol increases $T_{1\rho}^{-1}$ more than gramicidin, in part because cholesterol increases the static order parameter of the bilayer, thus making the ^{19}F-^{1}H cross-polarization more efficient. We have found that gramicidin causes the orientation dependence to change significantly for bilayers of protonated 2-[4,4-$^{19}F_2$]DMPC, compared to the dependence for pure 2-[4,4-$^{19}F_2$]DMPC bilayers (Peng et al., 1988). Under a locking field of 4 Gauss, the orientation dependence for the gramicidin-containing 2-[4,4-$^{19}F_2$]DMPC bilayers already resembles the orientation dependence predicted by the director-fluctuation model.

The effect of adding gramicidin is largest for the ^{19}F-label in the 2-[4,4-$^{19}F_2$]DMPC, and becomes progressively smaller as the ^{19}F-label moves toward the tail of the acyl chain. This particular label-position specificity can be explained by the presence of four bulky tryptophan residues near the carboxyl terminus of gramicidin. Because gramicidin forms antiparallel dimers with its amino terminus at the bilayer and midplane (Weinstein et al., 1979), ^{19}F-labels near the head group of the phospholipid should be affected most and both bilayer leaflets should be affected identically.

Cholesterol enhances the relaxation rate significantly for all the lipids studied without showing an obvious label-position dependence. This effect can be achieved by enhancing or inducing additional slow fluctuations of the whole phospholipid molecule. The disk-like shape of the cholesterol molecule may cause some microscopic defects to form around it upon its insertion into the bilayer, and open new possibilities for both fast and slow motions. The rigidity of the cholesterol molecule can explain, at least partially, the lack of a label-position specificity.

Relationship to nematic liquid crystals

Most theories being used to treat the nuclear relaxation in phospholipid bilayers are borrowed from the physics of liquid crystals (e.g., see de Gennes, 1974). Nevertheless, the orientation dependence of $T_{1\rho}^{-1}$ found in oriented phospholipid bilayers (Fig. 3 *A*) is quite different from that found in oriented, nematic liquid crystals (Doane et al., 1974). We believe that the difference is determined by their underlying structures. For most nematic liquid crystals, the shape of the molecule is close to that of a rigid rod. The molecule cannot move any portion in any direction without changing the orientation of the entire rod. The director fluctuation is apparently the only important slow motion in such a system. However, a phospholipid molecule has a flexible and segmented chain structure. Within certain global constraints, each segment can move more or less by itself. This seems to be the main reason that complicates their dynamic behavior, but also makes them physiologically useful as a component of a cell membrane. While the experimental results obtained for nematic liquid crystals can be well explained by the existing theories, more theoretical and experimental investigations are clearly required to explain the fluctuation and relaxation properties in phospholipid bilayers.

CONCLUDING REMARKS

In the following, we summarize our current understanding of slow molecular motions in phospholipid bilayers. We base our conclusions mainly on the results obtained for the 2-[7,7-^{19}F]DMPC-d_{52} oriented bilayers, because we believe that the interpretation of these experiments is the most straightforward one.

(*a*) No slow motions with a well-defined correlation time have been found in the region of 5×10^{-6} to 4×10^{-5} s. Slow motions apparently exist on this timescale only with a broad distribution of correlation times or exist at a shorter timescale. This situation is not affected by adding cholesterol or gramicidin to the bilayer.

(*b*) Models having either a single slow motion with an arbitrary geometry or a superposition of two specific types of slow motions (e.g., an anisotropic reorientation and a director fluctuation) can both be used to fit the observed orientation dependence of $T_{1\rho}^{-1}$. But it is impossible to identify uniquely the number of motions and to determine the geometry for each motion by using the orientation dependence measured at a single or in a small range of locking field strengths.

(*c*) The addition of cholesterol or gramicidin has a much larger effect on the slow motions in phospholipid bilayers than on the fast motions, as can be seen from the fact that $T_{1\rho}^{-1}$ is enhanced significantly in the presence of cholesterol or gramicidin, but T_1^{-1} is either unchanged or slightly decreased.

(*d*) The two additives have different label-position specificity. Gramicidin has its largest effect when the label is near the head group of the phospholipid, while cholesterol shows similar effects for all the label positions.

(*e*) Cholesterol and gramicidin show distinct effects on the orientation dependence of $T_{1\rho}^{-1}$. Cholesterol changes the orientation dependence toward the shape predicted for an anisotropic reorientation with $\langle P_2 \rangle = \langle P_4 \rangle$; grami-

cidin, when it is effective, changes the orientation dependence toward the shape predicted for collective director fluctuations.

We thank Dr. S. R. Dowd of our laboratory for synthesizing the phospholipids used in this research. We would like to thank Drs. M. Bloom, M. F. Brown, B. A. Cornell, I. J. Lowe, and J. F. Nagle for helpful discussions.

This work is supported by research grants from the National Institutes of Health (GM-26874 and HL-24525).

Received for publication 9 May 1989 and in final form 5 July 1989.

REFERENCES

Andersen, O. S. 1984. Gramicidin channels. *Annu. Rev. Physiol.* 46:531–548.

Bloom, M., and I. C. P. Smith. 1985. The use of deuterium NMR to study lipid-protein interactions in natural membranes. *In* Progress in Protein-Lipid Interaction. J. J. H. H. M. du Pont and A. Watts, editors. Elsevier Science Publishers, B. V., Amsterdam. 61–88.

Brown, M. F., A. J. Deese, and E. A. Dartz. 1982. Proton, carbon-13 and phosphorus-31 NMR methods for the investigation of rhodopsin-lipid interactions in retinal rod outer segment membranes. *Methods Enzymol.* 81:709–728.

Chupin, V., J. A. Killian, and B. de Kruijff. 1987. ^{2}H-Nuclear magnetic resonance investigations on phospholipid acyl chain order and dynamics in the gramicidin-induced hexagonal H_{II} phase. *Biophys. J.* 51:395–405.

Cornell, B. A. 1987. Gramicidin A-phospholipid model systems. *J. Bioenerg. Biomembr.* 19:655–676.

Cornell, B. A., and M. Keniry. 1983. The effect of cholesterol and gramicidin A′ on the carbonyl groups of dimyristoylphosphatidylcholine dispersions. *Biochim. Biophys. Acta.* 732:705–710.

Cornell, B. A., J. B. Davenport, and F. Separovic. 1982. Low-frequency motion in membranes: the effect of cholesterol and proteins. *Biochim. Biophys. Acta.* 689:337–345.

Cornell, B. A., R. G. Hiller, J. Raison, F. Separovic, R. Smith, J. C. Vary, and C. Morris. 1983. Biological membranes are rich in low-frequency motion. *Biochim. Biophys. Acta.* 732:473–478.

Davis, J. H., D. M. Clare, R. S. Hodges, and M. Bloom. 1983. Interaction of a synthetic amphiphilic polypeptide and lipids in a bilayer structure. *Biochemistry.* 22:5298–5305.

de Gennes, P. G. 1974. The Physics of Liquid Crystals. Oxford University, London.

Doane, J. W., C. E. Tarr, and M. A. Nickerson. 1974. Nuclear spin-lattice relaxation in liquid crystals by fluctuations in the nematic director. *Phys. Rev. Lett.* 33:620–624.

Dufourc, E. J., E. J. Parish, S. Chitrakorn, and I. C. P. Smith. 1984. Structural and dynamical details of cholesterol-lipid interaction as revealed by deuterium NMR. *Biochemistry.* 23:6062–6071.

Engelsberg, M., S. R. Dowd, V. Simplaceanu, B. Cook, and C. Ho. 1982. Nuclear magnetic resonance line-shape analysis of fluorine-19-labeled phospholipids. *Biochemistry.* 21:6985–6989.

Huang, C. 1977. A structural model for the cholesterol phosphatidylcholine complexes in bilayer membrane. *Lipids.* 12:348–356.

Jacob, R., and E. Oldfield. 1979. Deuterium nuclear magnetic resonance investigation of dimyristoyllecithin-dipalmitoyllecithin and dimyristoyllecithin-cholesterol mixtures. *Biochemistry.* 18:3280–3285.

Peng, Z.-y. 1988. Study of slow molecular motions in phospholipid bilayers. Ph.D. thesis. Carnegie Mellon University, Pittsburgh, PA.

Peng, Z.-y., V. Simplaceanu, S. R. Dowd, and C. Ho. 1989. Effects of cholesterol and gramicidin on slow and fast motions of phospholipids in oriented bilayers. *Proc. Natl. Acad. Sci. USA.* In press

Peng, Z.-y., V. Simplaceanu, I. J. Lowe, and C. Ho. 1988. Rotating-frame relaxation studies of slow motions in fluorinated phospholipid model membranes. *Biophys. J.* 54:81–95.

Pope, J. M., L. Walker, B. A. Cornell, and F. Separovic. 1982. A study of the angular dependence of NMR relaxation times in macroscopically oriented lyotropic liquid crystal lamellar phases. *Mol. Cryst. Liq. Cryst.* 89:137–150.

Shimshick, E. J., and H. M. McConnell. 1973. Lateral phase separations in binary mixtures of cholesterol and phospholipids. *Biochem. Biophys. Res. Commun.* 53:446–451.

Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science (Wash. DC).* 175:720–731.

Smith, R. L., and E. Oldfield. 1984. Dynamic structure of membranes by deuterium NMR. *Science (Wash. DC).* 225:280–288.

Tamm, L. K., and J. Seelig. 1983. Lipid solvation of cytochrome *c* oxidase. Deuterium, nitrogen-14, and phosphorus-31 nuclear magnetic resonance studies on the phosphocholine head group and on *cis*-unsaturated fatty acyl chains. *Biochemistry.* 22:1474–1483.

Weinstein, S., B. A. Wallace, E. R. Blout, J. S. Morrow, and W. Veatch. 1979. Comformation of gramicidin A channel in phospholipid vesicles: a ^{13}C and ^{19}F nuclear magnetic resonance study. *Proc. Natl. Acad. Sci. USA.* 76:4230–4234.

Yeagle, P. L. 1985. Cholesterol and the cell membrane. *Biochim. Biophys. Acta.* 822:267–287.