Molecular Motion in Phospholipid Bilayers in the Gel Phase: Long Axis Rotation†

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ABSTRACT: The molecular motions of a phospholipid spin-label have been studied in lipid bilayers below their ordered–fluid phase transition by using saturation transfer electron spin resonance, a technique which is sensitive to molecular motion in the correlation time range 10^{-7}–10^{-3} s. An upper limit of $\geq 10^{-4}$ s is placed on the effective correlation time of the lipid molecular motions in phosphatidylethanolamine bilayers below the main transition and in phosphatidylcholine bilayers below the pretransition. The cooperative onset of rapid long axis rotation of the lipid molecules, with an effective correlation time of $\sim 10^{-6}$ s, is detected at a temperature coinciding with, or immediately below, the calorimetric pretransition in phosphatidylcholine bilayers. In phosphatidylethanolamine bilayers, which do not show the calorimetric pretransition, rapid long axis rotation does not occur until the main transition. The onset of rapid long axis rotation is also detected in the conventional electron spin resonance spectra of a steroid spin-label in oriented bilayers of phosphatidylcholine. At the pretransition, the steroid molecule begins to rotate about its long molecular axis with a correlation time of $\sim 10^{-9}$ s. It is postulated that the onset of long axis rotation may trigger functional transitions in the membrane in much the same way as has been suggested for the chain fluidization at the main transition.

It is now commonly accepted that a substantial part of the lipid in biological membranes is present in the fluid state exhibited by synthetic phospholipids above their ordered–fluid phase transition [for a review, see, e.g., Marsh (1975)]. However, membranes are capable of existing with their lipids partially in the ordered or gel phase (Oldfield et al., 1972; Smith et al., 1979), and in particular it seems possible that the lipid directly associated with protein complexes in the membrane will be in a state of immobilization (but probably not order) more closely approaching that of gel-phase bilayers [see, e.g., Stier & Sackmann (1973) and Blaurock & Stoeckenius (1971)]. The latter possibility is of especial importance since the motional state of these particular lipid molecules may be responsible for the communication between the protein units of the complex and for maintaining the complex in a functionally active conformation.

For the above reasons we have examined the lipid motions in gel-phase phospholipid bilayers using spin-label saturation transfer electron spin resonance spectroscopy (STESR)[1] which is sensitive to lipid motions in the slow correlation time range: 10^{-7}–10^{-3} s (Hyde, 1978; Thomas et al., 1976). It is found that rapid rotation of the phospholipid molecules about their long molecular axes occurs above the pretransition in phosphatidylcholine bilayers, with a cooperative onset at a temperature coinciding with or immediately below that of the calorimetric pretransition. Similar effects are observed for intercalated steroid spin-label molecules by using conventional ESR spectra of oriented phosphatidylcholine bilayers. In phosphatidylethanolamine bilayers, which show no calorimetric pretransition, the lipid motions remain slow at all temperatures below the main ordered–fluid phase transition, having effective correlation times[2] $>10^{-4}$ s, of the same order of magnitude as are also found in the phosphatidylcholine bilayers below the pretransition.

These results demonstrate that rapid molecular motions can occur under conditions of lipid packing in which the chain segments remain substantially immobilized and any motion of the long axis of the molecule is also slow (i.e., effective correlation times $> 10^{-4}$ s). Such effects need not necessarily require that the lipids have a well-defined phase transition; it could also be the case for lipids in regions of high protein packing density as in specific protein complexes within the plane of biological membranes.

Experimental Section

Materials. All phospholipids (DMPC, DPPC, DLPE, and DMPE) were purchased from Fluka, Buchs, Switzerland, and the purity was checked by thin-layer chromatography. The 5-PCSL spin-label was prepared by Dr. A. Watts of this institute, according to the method of Hubbell & McConnell (1971). The CSL and 5-SASL spin-labels were purchased from Syva Associates, Palo Alto, CA. Formulas of the lipid spin-labels, with the orientation of the nitroxide axes, are given in Figure 1.

Sample Preparation. Aqueous phospholipid bilayer dispersions for STESR experiments were prepared by first mixing the lipid with 1–2 mol % spin-label in chloroform solution, evaporating off the chloroform with a stream of dry nitrogen, and then placing it under vacuum for at least 3 h. The dried lipid was then dispersed in 100 μL of buffer to a final concentration of 80 mM by vortex mixing at a temperature above the ordered–fluid bilayer phase transition. The dispersion was then sealed in a 1.2-mm o.d., 1.0-mm i.d. glass capillary for STESR measurement.[3] Oriented, planar phospholipid bilayers

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[1] Abbreviations used: STESR, saturation transfer electron spin resonance; DPPC, L-β,γ-dipalmitoyl-α-phosphatidylcholine; DMPC, L-β,γ-dimyristoyl-α-phosphatidylcholine; DMPE, L-β,γ-dimyristoyl-α-phosphatidylethanolamine; DLPE, L-β,γ-dilauryl-α-phosphatidylethanolamine; 5-PCSL, β-[5-(4′,4′-dimethyloxazolidinyl-*N*-oxy)stearoyl]-γ-palmitoyl-α-phosphatidylcholine; 5-SASL, 5-(4′,4′-dimethyloxazolidinyl-*N*-oxy)stearic acid; CSL, 4′,4′-dimethylspiro[5α-cholestane-3,2′-oxazolidin]-3′-yloxy; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; v_2' display, second harmonic of the ESR absorption detected 90° out-of-phase with respect to the field modulation.

[2] The motional parameters obtained from the STESR spectra of the lipid systems are referred to as *effective* correlation times since they are derived from calibration spectra corresponding to isotropic diffusion, whereas the lipid rotational diffusion is clearly anisotropic. This point is considered in more detail under Results and under Discussion.

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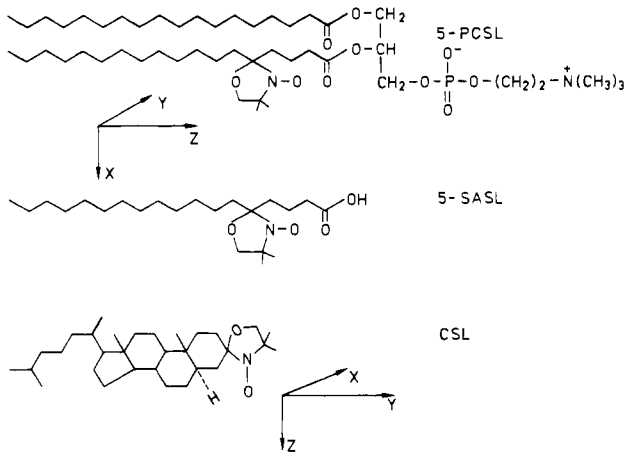

FIGURE 1: Spin-labels used, indicating the orientation of the nitroxide principal axes relative to the lipid long molecular axes. The principal values of the nitroxide hyperfine and g tensor are as follows: $A_{xx} \simeq A_{yy} = 5.8$ G; $A_{zz} = 30.8$ G; $g_{xx} = 2.0089$; $g_{yy} = 2.0058$; $g_{zz} = 2.0021$ (Hubbell & McConnell, 1971).

were formed by evaporating a chloroform solution of phospholipid plus 1 mol % spin-label onto the internal faces of a Varian E-248 quartz flat cell by using a stream of wet nitrogen. The flat cells were then placed under vacuum for at least 3 h and subsequently hydrated with aqueous buffer for 1–3 h at room temperature. The cells were drained and sealed immediately prior to measurement, ensuring that sufficient aqueous phase was retained to maintain complete hydration throughout the experiment. The buffer used for hydration was 0.1 M KCl–50 mM Tris, pH 8.0, in all cases.[4]

ESR Measurements. STESR spectra were recorded on a Varian E-109 Century Line spectrometer operating at 9 GHz and equipped with a quartz Dewar, nitrogen gas flow, temperature regulation system. Sample capillaries were contained within standard 4-mm quartz ESR tubes filled with silicone oil for thermal stability. Temperature was monitored by a thermocouple situated inside the ESR tube just above the microwave cavity. All STESR spectra were recorded in the second harmonic, 90° out-of-phase, absorption mode (v_2' display), with a modulation frequency of 50 kHz, modulation amplitude of 5 G, and microwave power of 63 mW. Measurements with peroxylamine disulfonate (Kooser et al., 1969) indicate that this corresponds to $H_1 \sim 0.25$ G for the sample arrangement used. The 90° out-of-phase setting was determined at microwave powers of 1 mW or less; out-of-phase nulls were in all cases ≤1% of the in-phase signal [see Marsh (1980) for further details]. Oriented bilayer spectra were recorded either with a Varian E-12 E-line 9-GHz spectrometer equipped with a nitrogen gas flow system for thermostating the entire microwave cavity (Thomas et al., 1976) or with a Varian V-4500 9-GHz spectrometer with a V-4535 large-access,

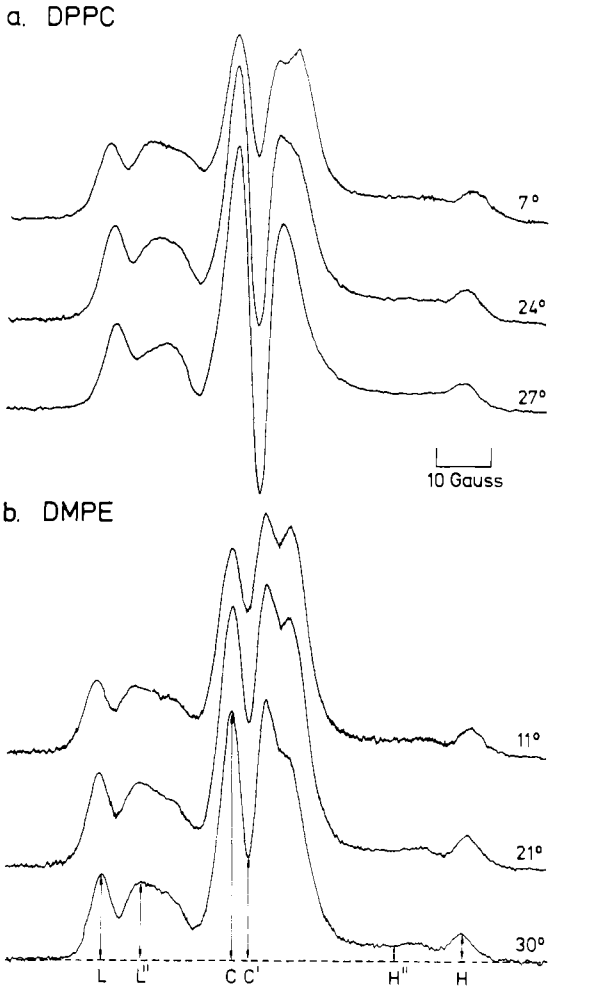

FIGURE 2: Second harmonic, 90° out-of-phase, absorption STESR spectra (v_2' display) of the 5-PCSL phospholipid spin-label in multibilayer dispersions of (a) DPPC and (b) DMPE as a function of temperature.

cylindrical cavity equipped with a large-diameter quartz Dewar capable of accommodating the E-248 flat cells. Temperature was measured by a fine-diameter thermocouple attached to the outer face of the flat cell, within the cavity. Spectra were recorded with the magnetic field oriented in the plane of the bilayer. The degree of orientation was checked by the anisotropy between these and the spectra with the magnetic field oriented perpendicular to the plane of the flat cell.

Results

STESR of 5-PCSL. The STESR spectra (v_2' display) of the 5-PCSL phospholipid spin-label in multilamellar dispersions of DPPC and of DMPE at various temperatures are given in Figure 2. Since the ordered–fluid phase transition temperatures of these two lipids are quite close (41 °C for DPPC and 48 °C for DMPE), it is valid to compare spectra from the two systems at similar temperatures in the gel phase. Clear differences are seen between the spectra of DMPE and DPPC in the direction of slower motion in the phosphatidylethanolamine. In particular, a sharp change in the central region of the DPPC spectra is observed at 25 °C, which is not seen with DMPE. Only small, gradual changes are seen for all parts of the DMPE spectrum throughout the whole temperature range below the main transition and similarly for the *outer* regions of the DPPC spectrum. These differential effects in the spectrum of DPPC bilayers are characteristic of the onset of a highly anisotropic motion in the gel phase and are

[3] The samples were normally incubated overnight at 4 °C before measurement, by which time the dispersions had settled out in the capillaries. It was generally found that freshly dispersed samples had lower out-of-phase/in-phase ratios and also lower C'/C ratios than those reported here. However, the same systematics in the change of line height ratios with temperature were observed for these freshly dispersed samples as those reported here for the incubated samples. No precautions were taken to exclude oxygen from the samples, which could shorten the apparent T_1 of the label. The out-of-phase/in-phase ratio, which is approximately proportional to T_1, was found to be of the order of 10% in nearly all cases.

[4] A maximum variation in pH of ±0.8 unit is expected over the temperature range studied. The pK values of the lipids studied lie well outside this range.

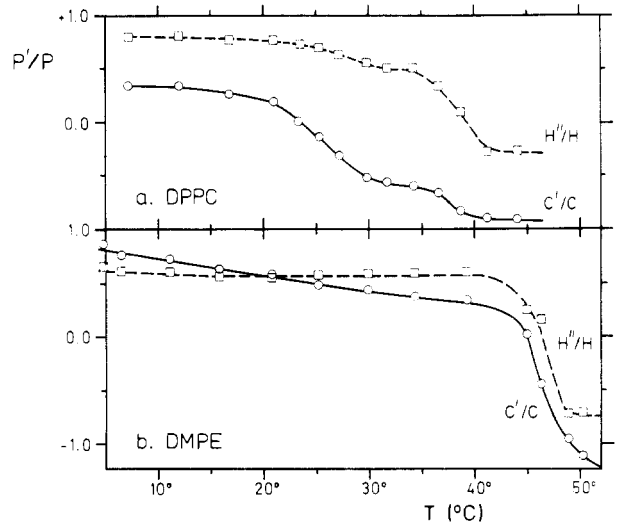

FIGURE 3: Central, C'/C, and high-field, H''/H, peak height ratios (see Figure 2) in the v_2' STESR spectra of (a) DPPC and (b) DMPE as a function of temperature.

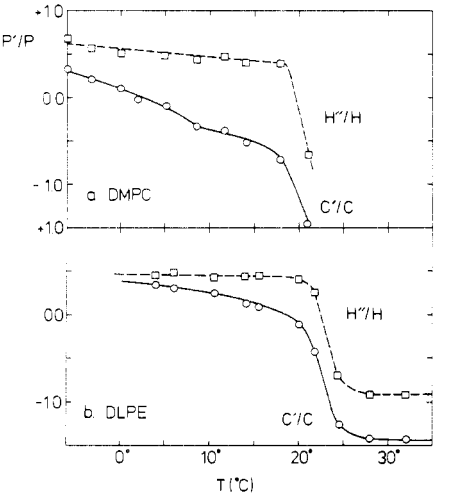

FIGURE 4: Central, C'/C, and high-field, H''/H, peak height ratios (see, e.g., Figure 2) in the v_2' STESR spectra of (a) DMPC and (b) DLPE as a function of temperature.

quantitated in Figure 3. This figure gives the temperature dependence of the diagnostic parameters H''/H and C'/C which are the peak height ratios used by Thomas et al. (1976) in the analysis of their spin-labeled hemoglobin reference spectra. H is the height of the peak at the high-field turning point, and H'' is the height at a point intermediate between the two high-field turning points. C and C' are similarly defined peak heights in the central region of the spectrum (see Figure 2). For simplicity, the low-field peak height ratio L''/L is not included in Figure 3, since this does not have a monotonically changing calibration with motional correlation time (Thomas et al., 1976).

From Figure 3a it is clear that there is an abrupt change in the parameter C'/C for DPPC at 25 °C whereas the change in the H''/H parameter is relatively small at this temperature. Both parameters show a change at the main transition, at 40 °C, the larger change being in the H''/H parameter. The transition in C'/C at 25 °C is interpreted as the onset of rapid rotation about the long axis of the phospholipid molecule with relatively little motion of the long axis itself. Reference to Figure 1 shows that rotation about the long molecular axis of the 5-PCSL molecules would modulate the anisotropy of the g tensor in the x–y plane, leaving all other tensor values unchanged (since the hyperfine tensor has approximate axial symmetry), hence only giving rise to saturation transfer in the central part of the spectrum. On the other hand, motion of the long axis itself would modulate all the spectral anisotropies giving rise to saturation transfer throughout the entire spectrum and in particular in the high-field and low-field regions. In contrast, it is seen that for DMPE in Figure 3b, both parameters H''/H and C'/C remain fairly constant right up to the main phase transition, and the onset of rapid rotation about the long molecular axis coincides with the fluidization of the chains at the main phase transition.

It was checked that the observed transition in C'/C corresponds to a change in motional correlation time and not to a change in spin–lattice relaxation time T_1 which could also affect the degree of saturation transfer and hence the peak height ratio. This was done by measuring the ratio of the amplitude of the 90° out-of-phase signal to that of the in-phase signal at a microwave power of 63 mW. This ratio is directly proportional to $\omega_m T_1$ (Thomas et al., 1976; Thomas & McConnell, 1974), where ω_m is the modulation frequency and thus can be used for detecting changes in T_1. In DPPC bilayers the out-of-phase to in-phase ratio remains approximately constant at 10–12%, and in DMPE it remains fixed below the phase transition at a value of 14–15%. The simulations of Thomas et al. (1976) show that changes within this range would have very little effect on the spectral parameters.

Table I: Effective Rotational Correlation Times of the 5-PCSL Spin-Label in Gel-Phase DPPC and DMPE Bilayers Deduced from Calibration of Peak Height Ratios of Hemoglobin Reference STESR Spectra[a]

	τ_2 (s)				
	12 °C	25 °C	30 °C	45 °C	50 °C
DPPC					
L''/L	6×10^{-4}	1×10^{-4}	0.8×10^{-4}	$\sim 10^{-9}$	
H''/H	4×10^{-4}	2×10^{-4}	1×10^{-4}	$\sim 10^{-9}$	
C'/C	0.8×10^{-4}	5×10^{-6}	0.9×10^{-6}	$\sim 10^{-9}$	
DMPE					
L''/L	2×10^{-4}	2×10^{-4}	2×10^{-4}	8×10^{-6}	$\sim 10^{-9}$
H''/H	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-5}	$\sim 10^{-9}$
C'/C	$\leq 10^{-3}$	10^{-4}	10^{-4}	6×10^{-6}	$\sim 10^{-9}$

[a] Reference spectra were taken from Thomas et al. (1976).

Similar effects are seen in the comparison of the STESR spectra of 5-PCSL in multilamellar dispersions of DMPC and DLPE (main phase transition temperatures at 23 and 29 °C, respectively) as indicated in Figure 4. A transition is observed in the C'/C parameter at 5 °C in DMPC whereas the H''/H parameter in DMPC and both parameters in DLPE remain almost constant up to the phase transition.[5] Again very little change is seen in the ratio of the out-of-phase to in-phase signal amplitudes which remains constant up until the main transition at 6–7% for DMPC and 9–10% for DLPE.

The above conclusions regarding long axis rotation are borne out when the effective rotational correlation times for isotropic motion are calculated from the peak height ratios by using the calibrations established from spin-labeled hemoglobin reference spectra by Thomas et al. (1976), as indicated in Tables I and II. It is implicit in these calibrations that if the motional rates about all the axes are the same, i.e., the motion

[5] The DMPC pretransition is not so well-defined as that for DPPC. This may in part be due to the fact that it is necessary to incubate DMPC at much lower temperatures to ensure that it is equilibrated well below its pretransition. Nonetheless, it is quite clear from Figure 4 that the temperature-induced change in C'/C is much greater than that of H''/H for DMPC or of either C'/C or H''/H for DLPE.

Table II: Effective Rotational Correlation Times Deduced from the STESR Spectra of the 5-PCSL Spin-Label in Gel-Phase Bilayers of DMPC and DLPE[a]

	τ_2 (s)				
	−5 °C	5 °C	10 °C	15 °C	25 °C
DMPC					
L''/L	3×10^{-4}	1×10^{-4}	0.9×10^{-4}	0.7×10^{-4}	$\sim 10^{-9}$
H''/H	2×10^{-4}	1×10^{-4}	0.8×10^{-4}	0.6×10^{-4}	$\sim 10^{-9}$
C'/C	2×10^{-5}	7×10^{-6}	3×10^{-6}	9×10^{-7}	$\sim 10^{-9}$
DLPE					
L''/L		1×10^{-4}	1×10^{-5}	1×10^{-4}	$\sim 10^{-9}$
H''/H		0.9×10^{-4}	0.9×10^{-4}	0.9×10^{-4}	$\sim 10^{-9}$
C'/C		5×10^{-5}	4×10^{-5}	4×10^{-5}	$\sim 10^{-9}$

[a] See the legend to Table I. Values are interpolated from Figure 4.

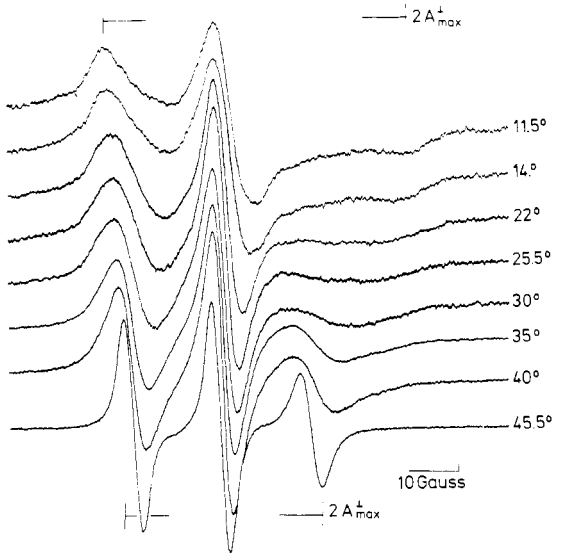

FIGURE 5: Conventional ESR spectra of the CSL steroid spin-label in oriented bilayers of DPPC, with the magnetic field oriented within the bilayer plane.

is isotropic, then all three peak height ratios should give the same correlation time (although with different sensitivities and precisions). Anisotropy of the motion can thus be detected by different effective correlation times being obtained from the different ratios [Marsh, personal communication in Hyde (1978) and Hyde & Dalton (1979)]. In Table I it is seen that for DMPE bilayers at all temperatures below the phase transition the effective motional correlation times are all approximately the same, in the region of 10^{-4} s. This is not the case, however, for DPPC: whereas the high-field and low-field peak height ratios give effective correlation times $\sim 10^{-4}$ s throughout the gel phase, the C'/C parameter indicates an abrupt change from $\tau_{eff} \sim 10^{-4}$ s to $\tau_{eff} \sim 10^{-6}$ s on passing through the transition at 25 °C. Thus, the rate of rotation about the long molecular axis increases by 2 orders of magnitude, whereas the change in motional rate of the long axis itself is at most a factor of 5. Similar differences between the gel phase motions in phosphatidylcholines and phosphatidylethanolamines are also seen in the comparison of DMPC and DLPE in Table II.

Conventional ESR of CSL. The conventional ESR spectra of the steroid CSL spin-label in oriented bilayers of DPPC, with the magnetic field oriented in the bilayer plane, are given in Figure 5. Because of the particular orientation of the nitroxide axes relative to the long molecular axis (the z axis is oriented perpendicular to the long axis and thus lies approximately within the bilayer plane; see Figure 1), these

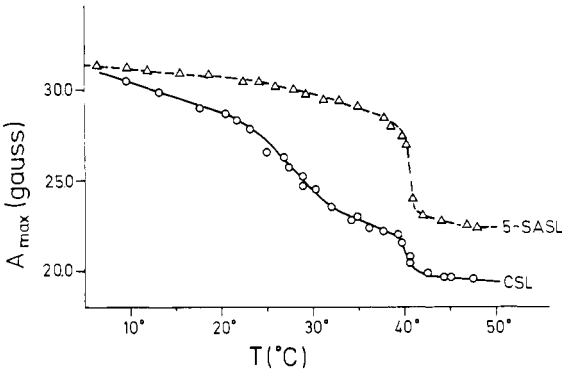

FIGURE 6: Maximum outer splittings (see Figure 5) of the conventional ESR spectra in DPPC oriented bilayers. (○) $A_{max}^{\perp}$ for CSL with the magnetic field in the bilayer plane. (△) $A_{max}^{\parallel}$ for 5-SASL with the magnetic field along the bilayer normal.

spectra should be optimally sensitive to long axis rotation of the molecule if the motional rate is sufficiently rapid. The spectra clearly indicate the change from an approximate two-dimensional powder spectrum at 11.5 °C with the z and x nitroxide axes oriented randomly and statically within the bilayer plane to a conventional, first-derivative, three-line spectrum at 35 °C, showing that all directions within the bilayer plane are then equivalent. The latter spectrum arises from the rapid rotation about the long axis which averages the hyperfine splittings in the x and z directions to give an axially symmetric splitting of $(1/2)(A_{xx} + A_{zz}) \simeq 19$ G. There is still a strong differential broadening of the lines at 35 °C, indicating that the correlation time for long axis rotation is $\gtrsim 10^{-9}$ s (Polnaszek, 1977). At 44.5 °C, above the main transition, there is a considerable narrowing of the spectra, indicating that the correlation time for the long axis rotation is then $\sim 2.10^{-10}$ s (Schindler & Seelig, 1974). The temperature dependence of this motional averaging of the hyperfine anisotropy by long axis rotation is indicated in Figure 6 which shows exactly the same pattern as that in the saturation transfer spectra of Figure 3a. The cooperative onset of long axis rotation is centered about a temperature of 27.5 °C, with complete rapid long axis rotation occurring in the region above 30 °C, while still in the phospholipid gel phase. Relatively little change occurs in the motion of the long axes of the molecules in the gel phase as indicated by the lack of motional averaging of the outer hyperfine splitting of the stearic acid spin-label, 5-SASL, in the same oriented bilayer system (see Figure 6). Consideration of Figure 1 shows that the spectra of this label, observed with the magnetic field oriented perpendicular to the bilayer plane (along the nitroxide z axis), should be optimally sensitive to rapid motion of the long molecular axis, which is seen not to occur until the main transition.

Similar effects to those of Figure 5 are seen in the conventional ESR spectra of the CSL label in random bilayer dispersions of phosphatidylcholines, although because of spectral overlap from the different orientations the cooperative nature of the onset of the long axis rotation is not nearly so clear. Spectral simulations in the slow motion time regime of the conventional ESR spectra of the CSL label have been made for such random dispersions by Polnaszek (1977), and empirical calibrations have been established. For values of the correlation time $\tau_R^{\parallel} \geq 10^{-9}$ s, the calibration parameter is the ratio A_{max}/A_{zz}, where $2A_{max}$ is the splitting between the two outermost peaks (cf. Figure 5). For the range 7.5×10^{-11} s $\leq \tau_R^{\parallel} \leq 10^{-9}$ s, the effective line width coefficients $B' = (1/2)\Delta H_0[(h_0/h_{-1})^{1/2} - (h_0/h_{+1})^{1/2}]$ and $C' = (1/2)\Delta H_0$-

Table III: Rotational Correlation Times of the CSL Spin-Label in Gel-Phase Bilayers of DPPC and DMPC, Deduced from the Line Splittings and Line Heights in the Conventional ESR Spectra[a]

	$\tau_R^{\parallel}$ (s)				
	4 °C	14 °C	24 °C	34 °C	44 °C
DPPC					
A_{max}/A_{zz}	$>10^{-8}$	4×10^{-9}	1.9×10^{-9}	1.2×10^{-9}	$\sim 10^{-10}$
B'	$>2 \times 10^{-9}$	$>2 \times 10^{-9}$	2×10^{-9}	0.4×10^{-9}	$\sim 10^{-10}$
C'	$>2 \times 10^{-9}$	$>2 \times 10^{-9}$	1.5×10^{-9}	0.2×10^{-9}	$\sim 10^{-10}$
	$\tau_R^{\parallel}$ (s)				
	5 °C	10 °C	15 °C	20 °C	25 °C
DMPC					
A_{max}/A_{zz}	$>10^{-8}$	7×10^{-9}	2.9×10^{-9}	1.4×10^{-9}	$\sim 10^{-10}$
B'	$>2 \times 10^{-9}$	$>2 \times 10^{-9}$	1.8×10^{-9}	0.6×10^{-9}	$\sim 10^{-10}$
C'	$>2 \times 10^{-9}$	$>2 \times 10^{-9}$	1.3×10^{-9}	0.2×10^{-9}	$\sim 10^{-10}$

[a] Calibrations were taken from spectral simulations by Polnaszek (1977); see also Marsh (1980).

Table IV: Effective Rotational Correlation Times of the CSL Spin-Label in Gel-Phase DMPC Bilayers Deduced from STESR Spectra[a]

	τ_2 (s)				
	−4 °C	0 °C	6 °C	11 °C[b]	17 °C[b]
L''/L	2×10^{-4}	1×10^{-4}	4×10^{-5}	$<10^{-7}$	$<10^{-7}$
H''/H	4×10^{-4}	4×10^{-4}	4×10^{-4}	9×10^{-5}	9×10^{-6}
C'/C	8×10^{-6}	5×10^{-6}	2×10^{-6}	1×10^{-7}	6×10^{-8}

[a] See the legend to Table I. [b] From 11 °C upward the in-phase spectra show indications of motional narrowing.

$[(h_0/h_{-1})^{1/2} + (h_0/h_{+1})^{1/2} - 2]$ are used for the calibration, where ΔH_0 is the peak-to-peak derivative width of the central line and h_{+1}, h_0, and h_{-1} are the peak heights of the apparent low-field, central, and high-field lines, respectively (Polnaszek, 1977; Marsh, 1980). The motional correlation times for rotation about the long axis of the CSL label in gel-phase DPPC and DMPC bilayers obtained by using these calibrations are given in Table III. From this it is again seen that for both DPPC and DMPC the correlation time decreases from a value $>10^{-8}$ s at low temperatures, well below the pretransition, to a value of $\sim 10^{-9}$ s at temperatures above the pretransition, but still in the gel phase below the main transition, and decreases further to values of 10^{-10} s above the main transition, in the fluid phase.

STESR of CSL. The STESR spectra (v_2' display) of the steroid spin-label in random multilamellar dispersions of DMPC were also studied in order to obtain an estimate of the rate of long axis rotation of the steroid molecules in gel-phase bilayers below the pretransition. An abrupt decrease was observed in the diagnostic line height ratios P'/P at temperatures approaching the pretransition, and above this temperature a motionally averaged second-derivative spectrum was observed, indicating rapid motion as observed above by conventional ESR. The effective motional correlation times obtained from the line height ratios of the STESR spectra in the temperature region below the pretransition are given in Table IV. At these temperatures all three parameters yield effective correlation times of $\sim 10^{-4}$ s, similar to those obtained for the 5-PCSL molecule below the pretransition. In this case, however, it is the H''/H and L''/L (and not the C'/C) ratios which give a measure of the rate of long axis rotation (cf. Figure 1).

Discussion

The above experiments demonstrate that rapid molecular motions are possible in ordered or gel-phase lipid bilayers, namely, long axis rotation in phosphatidylcholines above their pretransition. The lipid motions in the gel phase of phosphatidylethanolamines or phosphatidylcholines below the pretransition are considerably slower, with effective correlation times of $\sim 10^{-4}$ s. Such rates although slow are sufficiently rapid to be potentially significant for biological membrane function, when compared with enzyme turnovers or transport rates, for example. The effective values of 10^{-4} s must be considered as a lower limit because of the possible perturbing effect of the spin-label on the packing of these highly ordered bilayers. However, since much the same values are obtained for both the phosphatidylcholine and the steroid label (in contrast to the situation above the pretransition in phosphatidylcholines) and these labels have rather different molecular geometries, it seems probable that the motional rate may be limited by the tight packing in the bilayer rather than by the perturbing effect of the label. In addition, it is also likely that the slightly imperfect packing in the region of the probe might better approximate the situation in immobilized lipid regions of biological membranes since these will have a considerably heterogeneous lipid composition.

The application of STESR methods to these systems which have a well-defined molecular order, and in which the orientation of the nitroxide axes relative to the molecular axes is well-known, has lead to the unambiguous identification of a highly anisotropic motion in the slow correlation time regime [cf. D. Marsh [personal communication in Hyde (1978) and Hyde & Dalton (1979)]]. This conclusion is independent of any perturbing effect of the spin-label since a control is provided by the similar measurements in phosphatidylethanolamine bilayers, for which the motion is not present. The nature of this anisotropic motion has been further confirmed by the conventional ESR spectra of the steroid label, for which the rate of long axis rotation is faster. These results thus provide a set of calibration spectra for the analysis of STESR experiments in more complex systems in which the molecular orientation is less well-known. In general, for systems in which anisotropic motion is likely to occur, it is not to be expected that the effective correlation times derived from the different sets of diagnostic peak height ratios will be the same [cf. Baroin et al. (1977) and Kusumi et al. (1978)].

The observation of a cooperative onset of rapid long axis rotation in the gel phase of phosphatidylcholines but not of phosphatidylethanolamines correlates well with the existence of the calorimetric pretransition in phosphatidylcholines (Chapman et al., 1967; Hinz & Sturtevant, 1972) and the lack of a similar pretransition in phosphatidylethanolamines. The midpoint temperatures of the rotational transition found here are somewhat lower than those of the calorimetrically observed pretransitions. This is possibly due to the perturbing effect of the spin-label moiety, since the pretransition is known to

be particularly sensitive to the presence of impurities in the lipid phase.

The onset of rapid rotational motion is quite consistent with the other known properties of the phosphatidylcholine pretransition. Hinz & Sturtevant (1972) have pointed out that the enthalpy associated with the pretransition is of the order of magnitude expected from the onset of a rotational phase. Dilatometric measurements (Blazyk et al., 1975; Laggner & Stabinger, 1976) have indicated a cooperative increase in lipid partial specific volume at the pretransition which is again consistent with the onset of rapid rotational motion. X-ray diffraction studies (Janiak et al., 1976) have shown that a transition takes place in the symmetry of the chain packing from a distorted, closer-packed lattice below the pretransition to a hexagonal lattice above, in both DMPC and DPPC. The cylindrically symmetric packing above the pretransition would be consistent with long axis rotation of the molecules, but it must be noted that hexagonally symmetric chain packing is also observed in phosphatidylethanolamines in the gel phase (Harlos, 1978). Recent ^2H NMR studies on DPPC with perdeuterated hydrocarbon chains (Davis, 1979) have also detected the onset of long axis rotation of the lipid molecules. At −7 °C essentially all the molecules have stopped rotating on the deuterium NMR time scale, whereas at 20 °C there is an intermediate situation in which most of the molecules are exhibiting long axis rotation. For perdeuterated chains the situation is complicated by a possible partial segmental motion taking place in addition to the long axis rotation, and STESR studies on different PCSL positional isomers could shed some light on this.

The STESR definition of long axis rotation given here deserves some further comment. The motional averaging of the hyperfine tensor or the saturation transfer requires only that the x and z axes or the x and y axes, respectively, be interchanged for a maximal effect. This corresponds to a rotation of 90° rather than a complete 360° rotation. However, the symmetry of the situation probably dictates that the molecule will proceed forward a further 90° with at least equal probability to that with which it relaxes back after axis interchange. Hence, a full 360° rotation will be possible and follows directly from the existence of the 90° rotation. A further point regards the STESR calibration of the correlation time for long axis rotation using the standard spectra for isotropic rotation. In principle, isotropic motion will give rise to changes in the center of the spectrum from modulation of g_{zz} as well as the modulation of g_{xx} and g_{yy} which was proposed for the long axis rotation. However, the relative principal g values are such ($g_{xx} > g_{yy} > g_{zz}$; see legend to Figure 1) that the modulation of g_{xx} and g_{yy} will contribute most to the STESR central diagnostic region. Thus, the correlation times deduced from the C'/C ratio in Tables I and II will be a fair approximation to the correlation time for long axis rotation.

Finally, it is postulated that the cooperative onset of rapid long axis rotation may trigger functional transitions in much the same way as has been suggested previously for the chain fluidization at the main transition. Such rotational transitions may be less restrictive than the normal ordered–fluid-phase transitions, since it is possible that they may require neither the presence of a pretransition nor that the hydrocarbon chain composition be completely homogeneous. In this connection it is important to note that the cooperative onset of rapid long axis rotation has already been observed in chromaffin granule membranes (Fretten et al., 1980).

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References

Baroin, A., Thomas, D. D., Osborne, B., & Devaux, P. F. (1977) *Biochem. Biophys. Res. Commun. 78*, 442.

Blaurock, A. E., & Stoeckenius, W. (1971) *Nature (London), New Biol. 233*, 152.

Blazyk, J. F., Melchior, D. L., & Steim, J. M. (1975) *Anal. Biochem. 68*, 586.

Chapman, D., Williams, R. M., & Ladbrooke, B. D. (1967) *Chem. Phys. Lipids 1*, 445.

Davis, J. H. (1979) *Biophys. J. 27*, 339.

Fretten, P., Morris, S. J., Watts, A., & Marsh, D. (1980) *Biochim. Biophys. Acta* (in press).

Harlos, K. (1978) *Biochim. Biophys. Acta 511*, 348.

Hinz, H.-J., & Sturtevant, J. M. (1972) *J. Biol. Chem. 247*, 6071.

Hubbell, W. L., & McConnell, H. M. (1971) *J. Am. Chem. Soc. 93*, 314.

Hyde, J. S. (1978) *Methods Enzymol. 49*, 480–511.

Hyde, J. S., & Dalton, L. R. (1979) in *Spin Labeling: Theory and Applications* (Berliner, L. J., Ed.) Vol. 2, pp 1–70, Academic Press, New York and London.

Janiak, M. J., Small, D. M., & Shipley, G. G. (1976) *Biochemistry 15*, 4575.

Kooser, R. G., Volland, W. V., & Freed, J. H. (1969) *J. Chem. Phys. 50*, 5243.

Kusumi, A., Ohnishi, S., Ito, T., & Yoshizawa, T. (1978) *Biochim. Biophys. Acta 507*, 539.

Laggner, P., & Stabinger, H. (1976) in *Colloid and Interfacial Science* (Kerker, M., Ed.) Vol. V, pp 91–96, Academic Press, New York, San Francisco, and London.

Marsh, D. (1975) *Essays Biochem. 11*, 139.

Marsh, D. (1980) in *Membrane Spectroscopy* (Grell, E., Ed.) Springer-Verlag, Berlin, Heidelberg, and New York.

Oldfield, E., Chapman, D., & Derbyshire, W. (1972) *Chem. Phys. Lipids 9*, 69.

Polnaszek, C. F. (1977) *Int. Symp. Magn. Reson., 6th, Abstracts*, p 282.

Schindler, H., & Seelig, J. (1974) *J. Chem. Phys. 61*, 2946.

Smith, I. C. P., Butler, K. W., Tulloch, A. P., Davis, J. H., & Bloom, M. (1979) *FEBS Lett. 100*, 57.

Stier, A., & Sackmann, E. (1973) *Biochim. Biophys. Acta 311*, 400.

Thomas, D. D., & McConnell, H. M. (1974) *Chem. Phys. Lett. 25*, 470.

Thomas, D. D., Dalton, L. R., & Hyde, J. S. (1976) *J. Chem. Phys. 65*, 3006.