Saturation Transfer Electron Spin Resonance Spectroscopy as a Probe of Anisotropic Motion in Model Membrane Systems†

Michel Delmelle,‡ Keith W. Butler, and Ian C. P. Smith*

ABSTRACT: Saturation transfer electron spin resonance spectroscopy is applied to the study of slow anisotropic motions of the 5-doxylstearic acid and cholestane spin probes in model membranes of dipalmitoylphosphatidylcholine, in the presence (2:1 mole ratio) and absence of cholesterol, at temperatures below the usual gel to liquid-crystal phase transition of dipalmitoylphosphatidylcholine. In the case of multilamellar dispersions, the spectra were analyzed in terms of parameters used previously for isotropic motion of spin-labeled hemoglobin. Although these parameters vary considerably over the temperature range 0–40 °C, the spectra were significantly different from those observed previously for isotropic motion, and different spectral parameters yielded drastically different estimates of the correlation times. A more detailed view of the molecular dynamics was obtained from the spectra of oriented multibilayer films, where data could be taken at known angles between the axes of motional averaging and the applied magnetic field. A qualitative description of the motional processes in the rigid phase was obtained which agreed with conclusions reached by other methods; quantitative estimates of correlation times await the development of a satisfactory theoretical formalism for the influence of anisotropic motion in an ordered environment. Cholesterol was found to reduce the rigidity of the phospholipid acyl chains below the usual phase-transition temperature and to restrict mobility near this temperature. It is proposed that the oriented multibilayer system provides a more tractable test of beginning attempts at theoretical analysis.

A great deal of information about molecular dynamics of model and biological membranes has been acquired through the use of nitroxide spin-labels (Schreier et al., 1978; Berliner, 1976, 1979). Used conventionally, this technique is sensitive to molecular motions with rates greater than 10^7 s^{-1}. However, in order to obtain detailed insight into the various processes occurring in membranes, which appear to involve a wide range of correlation times, techniques which are sensitive to slower motions are urgently needed. Among other approaches, there have been recent efforts to develop saturation transfer methods in electron spin resonance (ST-ESR)[1] which should allow the measurement of correlation times in the range of 10^{-7}–10^{-3} s (Hyde, 1978; Hyde & Dalton, 1979; Dalton et al., 1976).

Thus far, however, ST-ESR has proven to be applicable only to the study of proteins, where it can be reasonably assumed that molecular motion is isotropic (Hyde & Thomas, 1973; Baroin et al., 1977, 1979; Hidalgo et al., 1978; Johnson, 1978; Kirino et al., 1978; Favre et al., 1979; Beth et al., 1979). Simulations have been confined to the case of isotropic motion (Thomas et al., 1976); theoretical and experimental results appear to agree reasonably well in this case.

Unfortunately, no satisfactory theoretical treatment has yet been proposed for the case of anisotropic motion, such as is normally encountered with lipids in membranes. Some experimental approaches have been undertaken where analysis was performed by using the isotropic data for reference. In particular, D. Marsh [personal communication in Hyde & Dalton (1979)] investigated two model membranes, DPPC and DMPE, with a spin-labeled PC. The results reveal the onset of an axial motion of the label occurring during the pretransition of DPPC, whereas this phenomenon was not observed with DMPE for which no pretransition is present.

In this study we approach the analysis of the spectra of complex anisotropic systems from a different viewpoint. Experiments were carried out with oriented multilamellar arrays of dipalmitoylphosphatidylcholine (DPPC)–cholesterol (2:1 molar ratio) mixtures containing either 5-doxylstearic acid (5-SASL) or cholestane spin probes (CSL). Since the orientation of the lamellar plane with respect to the magnetic field of the spectrometer is known, the ESR spectra obtained between 0 and 45 °C should reveal features which characterize specific modes of motion and, hence, provide information for the analysis of the spectra obtained with multilamellar dispersions.

The results of this investigation indicate that ST-ESR spectra are sensitive to the rates of anisotropic motion in membranes. Several parameters are proposed for the qualitative description of slow anisotropic molecular motion in these model systems.

Materials and Methods

Dipalmitoylphosphatidylcholine was purchased from Sigma Chemical Co., St. Louis, MO, cholesterol was from Steraloids Inc., Pawling, NY, and the 5-doxylstearic acid was from SYVA, Palo Alto, CA. The cholestane spin-label was synthesized by the method of Keana et al. (1967).

Sample Preparation. Chloroform solutions of dipalmitoylphosphatidylcholine or DPPC–cholesterol (2:1 molar ratio) were mixed with a chloroform solution of the spin-label, keeping the ratio of label to lipid less than 1:100. The solutions were evaporated with a stream of nitrogen, in a vial to form dispersions or in large quartz ESR cells (Scanco, Solvang, CA)

† From the Division of Biological Sciences, National Research Council, Ottawa, Canada K1A 0R6. *Received September 6, 1979.* NRC Publication No. 18052.

‡ Visiting NRC Scientist under the NATO Exchange Program. Present address: Département de Physique, Université de Liège, 4000, Sart-Tilman, Liège, Belgium.

[1] Abbreviations used: ST-ESR, saturation transfer electron spin resonance spectroscopy; DPPC, dipalmitoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; PC, phosphatidylcholine; 5-SASL, 5-doxylstearic acid, the 4,4-dimethyloxazolidinyl nitroxide derived from 5-ketostearic acid; CSL, the 4,4-dimethyloxazolidinyl nitroxide derived from 3-ketocholestane; ML-Hb, hemoglobin labeled with *N*-(1-oxy-2,2,6,6-tetramethyl-4-piperidinyl)maleimide (Thomas et al., 1976); ELDOR, electron–electron double resonance.

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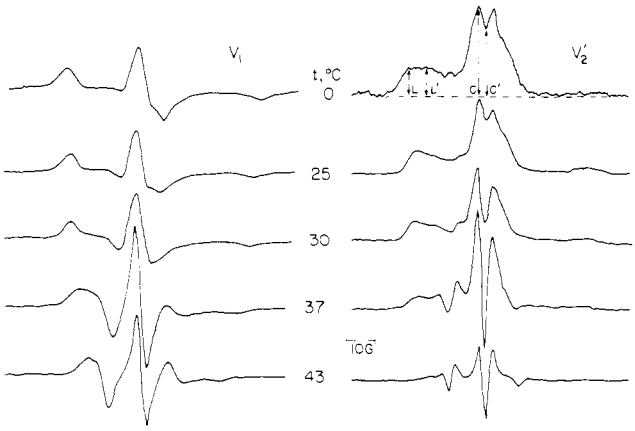

FIGURE 1: Electron spin resonance spectra, recorded at different temperatures, of DPPC multilamellar dispersions labeled with 5-SASL. Left side: first harmonic (100 kHz) in-phase absorption signals (derivative presentation) (V_1). Right side: second harmonic (200 kHz) 90° out of phase absorption signals (V_2'). The peak to peak modulation amplitude was 3.2 G, and the microwave power was 2 (V_1) and 120 mW (V_2').

to form films. The samples were exposed to a vacuum overnight to remove residual solvent and then hydrated with mammalian Ringer's solution (Altman & Dittmer, 1964). Samples were cycled through 45 °C before use. The structure of the labels, and the formation and use of planar films, has been discussed (Smith & Butler, 1976). The spectra of the dispersions were observed in variable-temperature cells (Scanco, Solvang, CA) in the Varian variable-temperature apparatus and of films were observed in a home-built insert (Cannon et al., 1975). The temperature was monitored with a thermocouple placed in the sample cell immediately above the active region of the cavity.

The ESR spectra were obtained with a Varian E9 ESR spectrometer modified to allow second harmonic observation, with accurate phase control, by means of Brookfield Ortholoc 9502 lock-in amplifier in place of the Varian receiver. V_1 (100-kHz modulation and observation) and V_2 (100-kHz modulation and 200-kHz observation) spectra were observed at 2-mW nominal power with the phase set for maximum signal amplitude. V_2' spectra were observed at 200 kHz with a receiver phase setting differing by 90° from that of the 100-kHz modulation. The spectrometer frequency was 9 GHz.

The phase for V_2' was set by interpolation between phases that gave V_2' spectra which are 10% of maximum amplitude on either side of the minimum. V_2' spectra were then recorded at 2-mW nominal power, which usually produced no signal intensity, and at 120 mW. This latter setting was chosen by matching the spectra from a lyophilized, maleimide spin-labeled hemoglobin sample (ML-Hb), run at various power levels, to the 63-mW spectrum published by Thomas et al. (1976). The modulation amplitude was set at 3.2 G to minimize overmodulation of the V_1 spectra and was calibrated by using the method described by Wertz & Bolton (1972).

As a check of the reporting fidelity of the fatty acid probe, spectra of dispersions were obtained by using dipalmitoylphosphatidylcholine with a nitroxide at the 5 position of the *sn*-2 fatty acyl moiety. No differences were observed between the V_2' spectra of this probe and those of 5-SASL.

Results

Figure 1 shows the V_1, and the corresponding V_2', ESR spectra observed with multilamellar dispersions of DPPC containing the spin probe 5-SASL. The V_1 spectra obtained between 0 and 30 °C cannot be analyzed by using the S parameter formalism, since the inner splitting is broadened beyond detection by slow motion (Cannon et al., 1975; Schreier et al., 1978). At 37 °C, this inner splitting becomes visible, although the line shape still suggests that the S parameter formalism is not applicable without serious error. At 43 °C, an order parameter can be estimated ($S = 0.44$), indicating that the model membrane is in the liquid-crystalline state at this temperature. Between 0 and 37 °C, the outermost splitting between the derivative extrema ($2T_{zz}'$) becomes progressively smaller. This indicates that some slow motion is taking place. A simple method for calculating the correlation time from the ratio of the observed splitting between the derivative extrema, $2T_{zz}'$, and the principal value of $2T_{zz}$ determined from the powder spectrum (64 G), assuming isotropic motion, has been proposed (McCalley et al., 1972; Goldman et al., 1975; Freed, 1976). Although this assumption is unrealistic in our case, we have used the value of the maximum splitting to estimate a correlation time (Table I).

Table I: Correlation Times for 5-SASL in Dispersions of DPPC Calculated by Using Isotropic Parameters

	τ (s)		
t (°C)	C'/C^a	L'/L^a	ΔT^b
0.5	$>10^{-3}$	$>10^{-3}$	$>10^{-6}$
7.2	$>10^{-3}$	10^{-4}	$>10^{-6}$
15	$>10^{-3}$	5×10^{-5}	10^{-7}
24.4	5×10^{-4}	5×10^{-5}	5×10^{-8}
30.4	8×10^{-5}	$<10^{-6}$	2×10^{-8}
34.7	4×10^{-6}	$<10^{-6}$	9×10^{-9}
37.6	2×10^{-6}	$<10^{-6}$	$<10^{-8}$

[a] Calculated via the calibration curves of Thomas et al. (1976). [b] Calculated via the method of McCalley et al. (1972).

The V_2' spectra in Figure 1 are very dependent on temperature. The 43 °C spectrum bears a resemblance to the V_2 spectrum (not shown), and we feel that, at this temperature, motion is too rapid to be analyzed from the saturation transfer spectra. At lower temperatures, however, the spectra resemble qualitatively those seen for proteins, and we consider them, as a first approximation, in terms of the parameters used for the case of isotropic motion. The C'/C parameter is obtained from the central region of the spectrum (Figure 1); C' measures the distance between the bottom of the valley in the central component and the base line, while C relates to the height of the left side of this central component. L'/L and H'/H (not measurable in Figure 1) relate to the low- and high-field components, respectively. L (H) measures the height of the peak near the low- (high-) field turning point, while L' (H') is the peak height at a point intermediate between the two low- (high-) field turning points in the V_1 spectra (Thomas et al., 1976).

The identification of the above parameters in our spectra is not unambiguous. The spectral intensity in the high-field region is very weak, and H'/H is virtually unmeasurable. The computation of C'/C is straightforward and the results are given in Table I. From these values, we computed the correlation times derived from the graphs published previously on the basis of spin-labeled hemoglobin [ML-Hb; Thomas et al. (1976)]. The parameter L'/L is not clearly defined; the spectra observed with 5-SASL in DPPC differ markedly from any published for ML-Hb. We measured L and L' at the two low-field maxima, insofar as the maxima were distinguishable (Figure 1). The corresponding correlation times, τ, are given in Table I. Between 0 and ~25 °C, the two parameters C'/C and L'/L reveal that the system is virtually rigid. In fact, the τ values derived from both spectral parameters fall at the limits of the published calibration curves (Thomas et al., 1976).

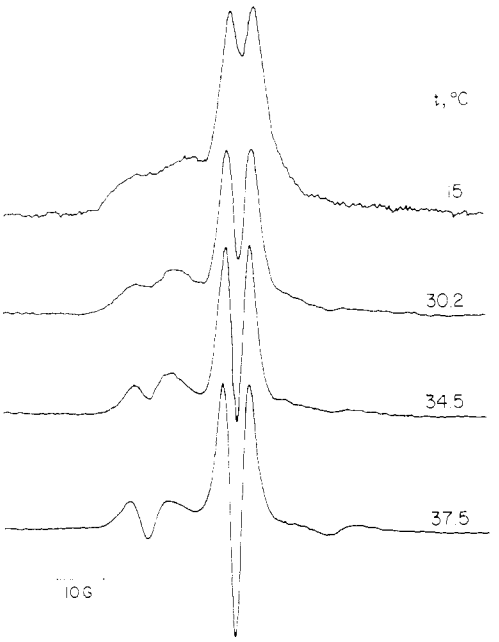

FIGURE 2: Second harmonic 90° out of phase ESR spectra (V_2') of DPPC dispersions labeled with CSL. Same recording conditions as in Figure 1.

Above 30 °C, a large discrepancy is observed between the values derived from the two parameters. L'/L indicates the onset of motion 1 to 2 orders of magnitude faster than that predicted by C'/C. This could be taken to suggest the onset of motions of the fatty acyl chain long axes, which involves the averaging of the $\mathbf{T}_{xx}$ and $\mathbf{T}_{zz}$, and $\mathbf{T}_{yy}$ and $\mathbf{T}_{zz}$, components of the hyperfine tensor, rather than axial rotations of the chains which average $\mathbf{T}_{xx}$ and $\mathbf{T}_{yy}$ and contribute mainly to C'/C. However, recent ^2H NMR studies suggest that the opposite is happening (Davis, 1979). Moreover, L'/L never becomes smaller than 0.65, corresponding to $\tau \approx 5 \times 10^{-5}$ s when motion is isotropic. In the isotropic case, L'/L approaches 0 as τ approaches 10^{-6} s. Finally, if one compares the values computed from V_1 and V_2' spectra, the differences are so large that the calibration technique becomes highly questionable. Both methods (from the V_1 and V_2' spectra) relate to isotropic motion, and the molecular dynamics of 5-SASL in DPPC are certainly anisotropic. The definition of the L'/L parameter itself should be questioned. This latter point becomes even more apparent in the case of DPPC labeled with CSL (Figure 2). In this case the L'/L parameter never becomes smaller than 1, between 0 and 37 °C. This is completely off the published calibration scale on the slow motion side (Thomas et al., 1976) while the C'/C parameter varies from 0.78 to −0.72, corresponding to τ values from 10^{-3} to 10^{-6} s.

It can be concluded from these experiments that although the V_2' spectra vary significantly in a temperature domain where the corresponding V_1 spectra are no longer sensitive, the parameters which have been used to analyze isotropic spectra do not apply simply. It is necessary to define other parameters in the ST-ESR spectra which are sensitive to anisotropic motion and to calibrate these parameters with respect to motional rates.

Effect of Cholesterol. The addition of 33 mol % cholesterol has been found to abolish essentially the phase transition of DPPC (Ladbrooke et al., 1968). As in the case of DPPC–5-SASL dispersions, the inner high-field peak of the V_1 spectrum is not resolved below 36 °C. At 37.5 °C, the turning point of the inner high-field line is on the base line, indicating a correlation time of about 5×10^{-9} s (Mason et al., 1974).

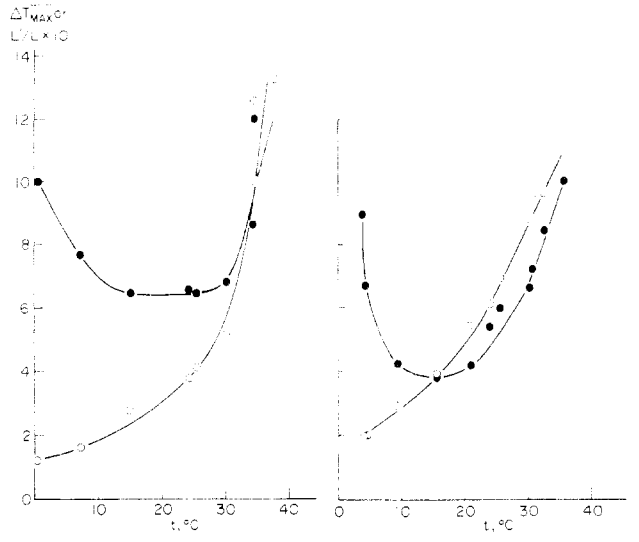

FIGURE 3: Temperature dependences of $\Delta\mathbf{T}_{max}$ and L'/L of 5-SASL in dispersions of DPPC (left) and DPPC–cholesterol (right). Open circles relate to ΔT_{max}, i.e., $2(\mathbf{T}_{zz} - \mathbf{T}_{zz}')$ measured from V_1 spectra; solid circles relate to L'/L values measured from the V_2' spectra. The scale is the same for both parameters.

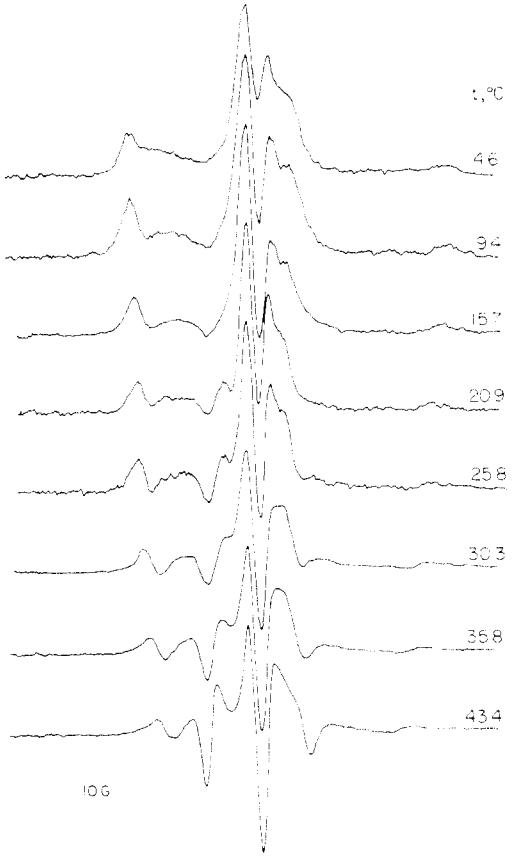

FIGURE 4: Second harmonic 90° out of phase ESR spectra of DPPC–cholesterol multilamellar dispersions labeled with 5-SASL at the indicated temperatures.

The maximum splittings observed in the V_1 spectra were temperature dependent. The difference between the observed outer splitting and $\mathbf{T}_{zz}$ is a measure of motion (vide supra). Up to 30 °C, this difference was smaller for 5-SASL in DPPC (Figure 3, left) than in DPPC–cholesterol (Figure 3, right), and the value increased more slowly with temperature. At temperatures above 30 °C this value increased more rapidly in DPPC than in DPPC–cholesterol. Thus, this parameter indicates that cholesterol mobilizes gel-state lipids whereas it

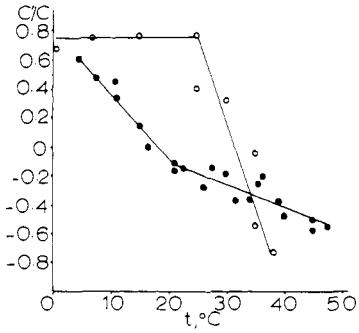

FIGURE 5: Temperature dependence of C'/C in dispersions of DPPC (open circles) and DPPC–cholesterol (solid circles) containing CSL.

restricts the mobility of liquid-crystalline lipids. This conclusion is in agreement with earlier calorimetry and X-ray studies (Ladbrooke et al., 1968) and permeability measurements (DeGier et al., 1969). The saturation transfer spectra (Figure 4) for 5-SASL also indicated that the presence of cholesterol reduced the rigidity of the system at low temperatures. The value of L'/L changed more for the mixed system than for pure DPPC (Figure 3) but did not go below ~0.4. It is further indication of the inapplicability of the isotropic formalism that, in both systems, increasing rates of motion led to minima in L'/L, both of which are greater than that predicted from the ML-Hb spectra (~0.1).

The value of C'/C, relative to data from spin-labeled hemoglobin, indicated that DPPC was completely rigid below 15 °C, while the rate of motion was measurable from C'/C with the addition of 33 mol % cholesterol. The value of C'/C remained greater in the absence of cholesterol up to 37 °C.

When the CSL label was used in DPPC–cholesterol mixtures, the spectral features look very similar to those shown in Figure 2. The L'/L parameter was greater than any value observed for isotropic motion. As measured by C'/C (Figure 5), cholesterol reduced the rigidity of the system at temperatures below the gel–liquid-crystal transition of DPPC and increased it at higher temperatures.

In order to get a better understanding of the spectral changes observed, we turned our attention to oriented multibilayer systems.

Oriented Multibilayers. Oriented multibilayers provide greater understanding of both spatial and dynamical properties of their component lipids than do the corresponding dispersions (Smith & Butler, 1976). These preparations have been extensively studied with conventional ESR technique because they provide a way to observe, on a macroscopic scale, a well-defined orientation of the nitroxide principal axes with respect to the applied magnetic field. The steroid spin probe has the major component of its hyperfine tensor ($\mathbf{T}_{zz}$) perpendicular to the long molecular axis, while in 5-SASL $\mathbf{T}_{zz}$ lies parallel to the fatty acid chain. Thus, the two spin probes can be considered as complementary. Since it is difficult to make films with DPPC alone, the following experiments were conducted with films of DPPC–cholesterol at a molar ratio of 2:1.

(A) CSL. Figure 6 presents some typical spectra obtained at 36 °C with DPPC–cholesterol films containing CSL. In the upper part of the figure, the V_1 spectra of the film with its plane parallel and perpendicular to the applied magnetic field of the spectrometer are superimposed. The latter spectrum is composed of three narrow lines separated by ~6 G. The splitting $2\mathbf{T}_\perp$ remains virtually constant over the 0–40 °C temperature range (Figure 7, left). The spectrum in the parallel orientation is considerably broader; the outermost

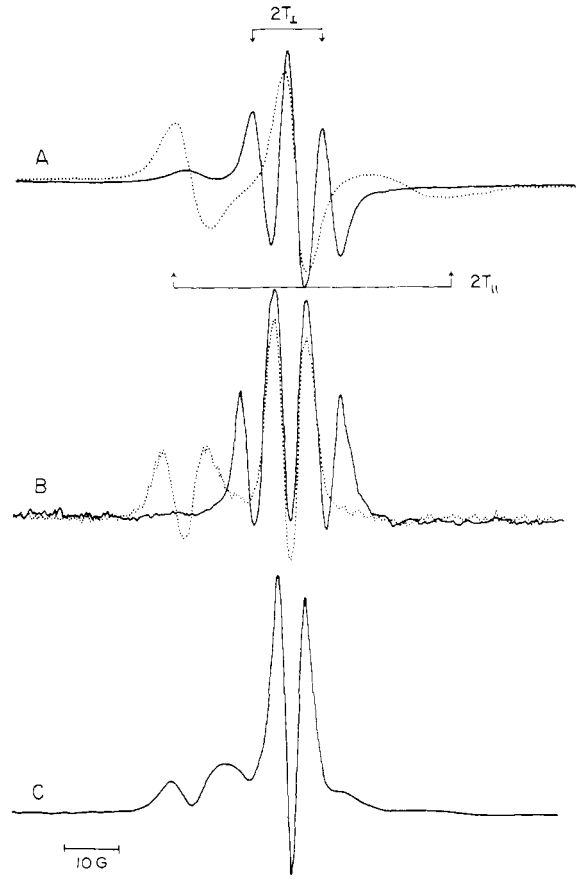

FIGURE 6: ESR spectra of DPPC–cholesterol systems, labeled with CSL, at 36 °C. (A) V_1 signal from films perpendicular (solid line) and parallel (dotted line) to the spectrometer magnetic field. (B) V_2' signal from perpendicular (solid line) and parallel (dotted line) film orientations. (C) V_2' signal from a DPPC–cholesterol dispersion.

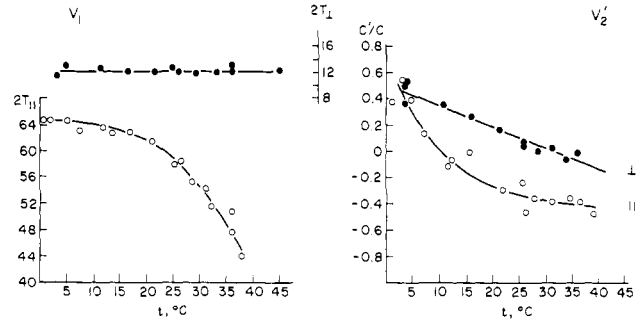

FIGURE 7: Temperature dependence of V_1 (left) and V_2' (right) spectral parameters from DPPC–cholesterol films labeled with CSL. Left: $2\mathbf{T}_\parallel$ and $2\mathbf{T}_\perp$ relate to films parallel and perpendicular to the external magnetic field (see Figure 6). Right: C'/C vs. temperature in perpendicular (closed circles) and parallel (open circles) film orientations.

separation between the spectral extrema ($2\mathbf{T}_\parallel$) is 47.7 G at 37 °C, which implies that the $\mathbf{T}_{zz}$ and $\mathbf{T}_{xx}$ components of the hyperfine tensor are partially averaged.

The values of $2\mathbf{T}_\parallel$, as a function of temperature, are shown in Figure 7 (left side). Below 20 °C, powder spectra are observed, and the corresponding $2\mathbf{T}_\parallel$ values are close to the theoretical maximum, 64 G. These spectra result from an envelope of spectra corresponding to all the orientations from $\mathbf{T}_{xx}$ to $\mathbf{T}_{zz}$ parallel to the applied magnetic field. This indicates a cessation of rotation of the probe about the steroid long axis on the V_1 time scale, i.e., slower than 10^7 s^{-1}. At and above 20 °C, however, $2\mathbf{T}_\parallel$ decreases, indicating partial motional averaging of $\mathbf{T}_{xx}$ and $\mathbf{T}_{zz}$. These data are in agreement with

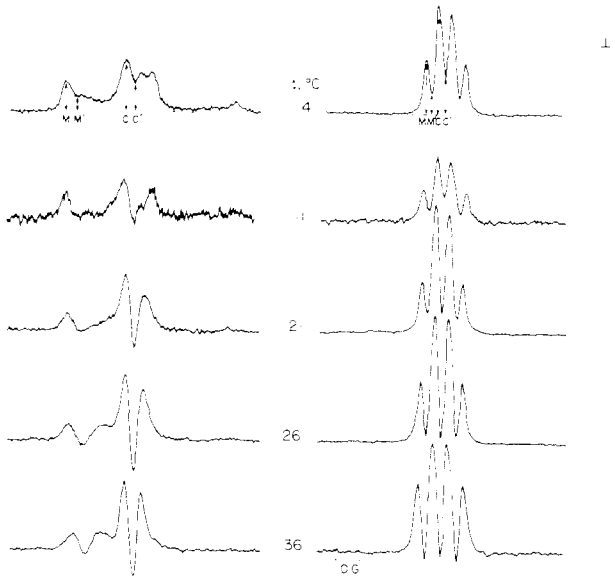

FIGURE 8: Second harmonic 90° out of phase spectra of DPPC–cholesterol films labeled with CSL, parallel (left) and perpendicular (right) to the magnetic field, at the temperatures indicated.

those obtained by Shimoyama et al. (1978) for the CSL probe in multibilayer films of DPPC, 30 mol % cholesterol, of somewhat lower water content.

Figure 6B shows the V_2' spectra at 36 °C, in both the parallel and perpendicular orientations of the films, while Figure 6C shows the spectrum of the corresponding multibilayer dispersion.

Figure 8 shows the dependence of the V_2' spectra on temperature. In the parallel orientation, one observes with increasing temperature a gradual shift of the outer components toward the center of the spectrum; this occurs simultaneously with a deepening of the valleys. In the perpendicular orientation, only this latter phenomenon is observed. This is consistent with the fact that in this temperature range, the $2\mathbf{T}_\perp$ splitting remained constant, indicating a minimal change in the degree of ordering of the probe and, hence, of y–z averaging (motion normal to the CSL long axis).

The main features of the V_2' spectra can be characterized by the valleys which appear at field positions close to the maxima of the corresponding absorption spectra. This observation bears some analogy with those made previously with spectra of dispersions. In the following, we will use as working parameters the distances from the bottom of a valley to the base line. For the central part of the spectrum, this obviously corresponds to the C'/C parameter defined earlier for isotropic motion.

The right-hand side of Figure 7 shows the evolution of C'/C [measured from the V_2' central component (Figure 8)] vs. temperature in the case of the data from the perpendicular and parallel orientations. In the former case, C'/C decreases linearly with temperature while with the parallel orientation, a large decrease of C'/C occurs between 5 and 20 °C. In this temperature range, the corresponding V_1 spectra did not change significantly. By contrast, when $2\mathbf{T}_\parallel$ starts to decrease in the V_1 spectra, i.e., above 20 °C, the C'/C values of the V_2' spectra tend to level off. The parameter M'/M (Figure 8) followed the behavior of C'/C in all cases. This comparison between the V_1 and V_2' data reveals some complementarity between the two modes of spectra acquisition; i.e., above 25 °C x–z averaging occurs rapidly enough to affect the hyperfine splitting; at these temperatures the motion is too rapid to produce effects in ST-ESR spectra.

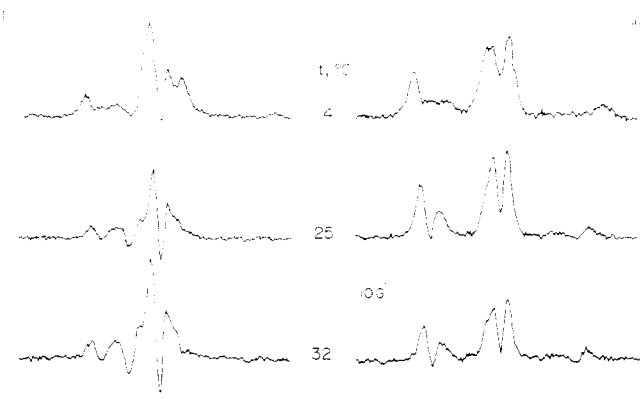

FIGURE 9: Second harmonic 90° out of phase spectra of a film of DPPC–cholesterol containing 5-SASL. Left: parallel to the magnetic field. Right: perpendicular to the magnetic field.

Indeed, the saturation transfer mode extends significantly the potentiality of the ESR technique to measure motion slower than 10^{-7} s. At 0 °C, both the V_1 ($\parallel$ and $\perp$) spectra and the V_2' spectra show that the system is very rigid. Between 0 and 20 °C, motion is detected only with the ST-ESR spectra. Moreover, the motion about the steroid axis which is detected from the V_2' ($\parallel$) spectra is faster than that in the perpendicular direction [V_2' ($\perp$)]. Hence, $\tau_\parallel$ should be smaller than $\tau_\perp$, as has been found consistently in simulations of the V_1 spectra of this probe (Cannon et al., 1975; Neal et al., 1976; Shimoyama et al., 1978). At 20 °C, the motion of the steroid molecule around its long axis becomes visible in the V_1 spectra. Simultaneously, the ST technique becomes less sensitive, since the M'/M and C'/C values level off. This temperature corresponds probably to the limit of sensitivity of the two approaches, suggesting that at 20 °C, $\tau \simeq 10^{-7}$ s.

From the spectra in the perpendicular orientation, information can be obtained about the motion of the steroid molecule orthogonal to the long molecular axis. It corresponds to the wobbling of the steroid molecule within a cone (Lapper et al., 1972). At 2 °C (not shown), the V_1 and V_2' spectra are close to the rigid limit. The steroid probe is probably not moving faster than 10^4 s^{-1} about any axis at this temperature. Futhermore, over the entire temperature range 0–40 °C, $\mathbf{T}_\perp$ remains virtually constant and close to the theoretical limit of 6 G, which implies that the wobbling motion is undetectable on the V_1 time scale and that its amplitude must be very small. However, the V_2' data reveal that in this temperature range some slow tumbling is taking place. The correlation time, $\tau_\perp$, probably varies from $\approx 10^{-4}$ s (0 °C) to 10^{-6}–10^{-7} s near 40 °C. The spectra of dispersions are the sums of those from all possible orientations of the bilayer normal with respect to the applied magnetic field. Therefore, the C'/C parameter should reflect a superposition of the two phenomena observed with the perpendicular and parallel orientations of the films. Figure 5, a plot of C'/C vs. temperature of dispersions, shows that this is indeed the case, since between 0 and $\simeq$20 °C the C'/C value decreases significantly faster than at higher temperatures. This compares with the observation made for the films in the parallel orientation. In the spectra of dispersions, the perpendicular component is less important than its parallel counterpart due to the nature of geometric averaging over a sphere.

(*B*) *5-SASL.* Figure 9 shows V_2' spectra of 5-SASL in a multibilayer film of DPPC–cholesterol, oriented parallel (left) and perpendicular (right) to the applied magnetic field. The lowest field components of the V_2' spectra originate from nitroxide molecules whose long axes are not oriented perpen-

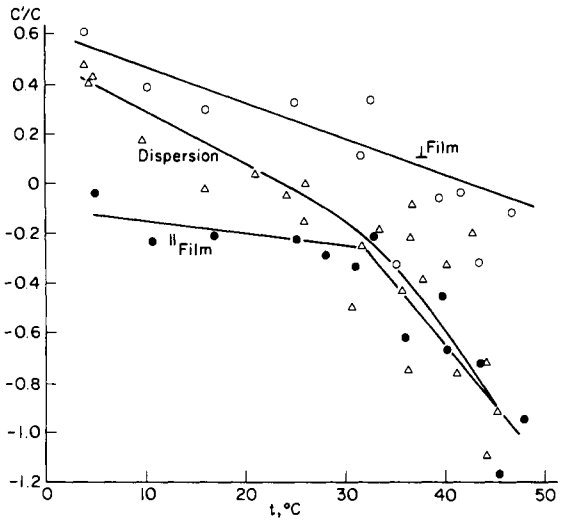

FIGURE 10: Comparison of the temperature dependence of C'/C in DPPC–cholesterol mixtures containing 5-SASL, in parallel and perpendicular film orientations and in multilamellar dispersions.

dicularly to the applied magnetic field. By contrast, the quality of the $V_1(\perp)$ and $V_2'(\perp)$ spectra remains good even at low temperatures. The V_2' spectra of 5-SASL have features which can be compared with those observed with CSL, namely, the presence of peaks at field positions which match with the maxima of the corresponding absorption peaks. Each of these peaks is split by a valley which tends to become deeper as temperature increases (Figure 9). In the $V_2'(\perp)$ spectra, the three peaks are further apart than in the $V_2'(\|)$ spectra. The same phenomenon is observed, as expected, in the V_1 spectra, since in 5-SASL the largest component of the hyperfine tensor lies parallel to the long molecular axis. With this spin probe, the T_{zz} component should be sensitive to the wobbling motion of the fatty acid molecule.

With CSL no wobbling motion of the probe was detected between 0 and 40 °C on the V_1 time scale, since the inner splitting remained virtually constant. On the contrary, in the case of 5-SASL, $2T_\perp$ decreases slowly from 64 G at 4 °C to ~55 G at 40 °C. The $V_2'(\perp)$ spectra provide some additional information. They reveal that motion is taking place on the V_2' time scale, implying that T_{zz} is wobbling at a rate slower than 10^7 s^{-1}. Indeed, $C'/C(\perp)$ varies from ~0.6 at 0 °C to ~0.2 at 40 °C. The evolution of C'/C vs. temperature compares with the data from the CSL films in the perpendicular orientation.

(C) Comparison of Results for Films and Dispersions. Figure 10 compares the values of C'/C for 5-SASL in films and dispersions of DPPC–cholesterol (2:1). The dispersion values lie between those obtained with the film parallel and perpendicular to the magnetic field of the spectrometer and approach the parallel values at high temperature. Similar results were obtained with films and dispersions labeled with CSL (compare Figures 5 and 7).

Discussion

For several years, various efforts have been made to extend the use of ESR to monitor the slow motions of macromolecules, i.e., motion characterized by correlation times in the millisecond to microsecond range; a typical membrane protein such as rhodospin has been shown to have a rotational correlation time of 20 μs (Cone, 1972). The conventional ESR technique does not allow the measurement of such long correlation times since its sensitivity peaks around 10^{-9} s. Goldman et al. (1972) and McCalley et al. (1972) have proposed a method which extends the technique by ~2 orders of magnitude through a careful analysis of the shape of the V_1 spectra. Their interpretation is based on the assumption that the motion is isotropic.

Other methods to measure slow motions with ESR are referred to as saturation transfer because the response is strongly affected by the diffusion of saturation between different portions of the spectrum. This diffusion is dominated by motional modulation of the **g** and hyperfine tensors. ELDOR is the most straightforward approach (Smigel et al., 1974) but is complex experimentally. The dispersion method, which implies a 90° out of phase detection with respect to the field modulation, has a low detection sensitivity. At present, it appears that the absorption mode, with 90° out-of-phase detection with respect to the second harmonic of the field modulation, will be most commonly used, although interpretation of the data is probably more complex than for the dispersion mode (Hyde & Dalton, 1979). The application of the saturation transfer absorption method to the study of spin-labeled hemoglobin in aqueous glycerol solution (Thomas et al., 1976) constitutes the calibration of most of the subsequent applications of the saturation transfer technique. It provides a convenient methodology for the interpretation of the ST-ESR spectra by establishing a correlation between the spectra features and rotational correlation times deduced from the Stokes–Einstein–Debye equation. Theoretical analysis has provided simulated spectra (Thomas et al., 1976).

Various efforts are currently underway to extend the potentiality of the ST-ESR technique to problems involving anisotropic motion. The theoretical approach is unquestionably very involved, and so far no satisfactory treatment has been published. This paper provides an experimental approach to interpretation of ST-ESR spectra. The rationale is to simplify as much as possible the experimental system and, hence, to get a better understanding of the observed spectra. The oriented multibilayers allow one step in this direction since they provide the opportunity to observe, on a macroscopic scale, one or two oriented components of the hyperfine tensor. The spectra obtained with such preparations look very promising, since they are characterized by resolvable features which are sensitive to the evolution of the V_2' absorption spectra in the slow motion regime and involve only one or two components of the hyperfine tensor. One of the major goals of the present work is to stimulate future research in anisotropic systems; it is felt that the data obtained with the oriented films could provide a tractable system for detailed theoretical analysis.

Should this new technique become quantitatively useful, it would provide an important adjunct to other approaches such as NMR and fluorescence spectroscopy. On the other hand, the present work demonstrates that the use of isotropic parameters in the analysis of spectra for anisotropic systems can be very misleading not only in the estimation of correlation times but also in the qualitative conclusions. The definition of the parameter L'/L and H'/H does not apply to the spectra observed with 5-SASL and CSL spin-labels. The fact that our experiments were performed at 200 kHz while the hemoglobin spectra were obtained at 100 kHz does not invalidate the present conclusions; this frequency difference should only shift the reference curve slightly.

The present results for the gel phases of DPPC and DPPC–cholesterol allow a qualitative description of the molecular dynamics. For quantitative calibration of the spectra in terms of absolute correlation times, two approaches seem feasible. One would be to use other experimental techniques which provide reliable information concerning the order pa-

rameter and the various correlation times involved. In this respect, ^2H and ^{13}C NMR are promising. The other approach would be to simulate adequately the experimental spectra using convenient computer programs which take into account at least one order parameter and two correlation times. The effects of relaxation times T_1 and T_2 should also be a major concern.

Our present description is based on the use of simple empirical parameters which are actually just a generalization of the C'/C parameter used in experiments on isotropic systems. Although the conclusions we have reached concerning molecular motion are in agreement with the present knowledge of the lecithin gel phase, future application of the ST-ESR technique calls for quantitative determinations of the various motional modes.

Several techniques led to the commonly accepted model for hydrated lecithin in the gel phase where the molecules are thought to be in an ordered state, rotating rapidly about the long molecular axis and hexagonally packed with the hydrocarbon chains in mainly the all-trans configuration (Davis, 1979). Our data support this general scheme: the observation of ST-ESR spectra between 0 and 40 °C indicates that slow motions are present. The difference between DPPC and DPPC–cholesterol mixtures reveals an effect of cholesterol to increase the degree of lipid mobility at temperatures below that of the phase transition of DPPC. A vestige of the DPPC phase transition remains detectable in the mixture containing 33% cholesterol, by both calorimetric and spin-label experiments. Our data for these systems at low temperature ($\sim$0 °C) are consistent with the existence of a rigid lattice with very restricted and slow motion. This conclusion was reached with both the CSL and 5-SASL spin-labels: however, in DPPC–cholesterol mixtures some axial rotation is detectable for 5-SASL at low temperature, whereas CSL does not appear to undergo such motion. Between 0 and 30 °C, the onset of motion of CSL begins. The rate of axial rotation is always faster than that of the wobbling motion of the probe long axis. In the "pretransition" region (36 °C) the rate of wobbling of 5-SASL becomes rapid and measurable in the V_1 spectra.

In summary, the present study demonstrates the usefulness of ST-ESR spectra of anisotropic model membrane systems. The use of oriented films contributes a greater understanding of the ST-ESR spectra themselves. It is hoped that a firm theoretical basis for the quantitative interpretation of these spectra will soon become available.

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