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Anisotropic Motion of Lipids in Hydrophobic Core of DPPC Bilayers. Simulation of ESR spectra

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Abstract

Planar samples prepared by drying phospholipid dispersions on glass slides provide a convenient model to examine motion and orientation in lipid bilayer structures. In the present work, hydrophobic cores of DPPC multibilayers were studied using a 16-DSA spin label consisting of a nitroxide free radical bonded to the 16 position of stearic acid. This spin label is in great demand for investigation of phase transitions occurring in the hydrophobic cores of lipid multibilayers. Thus, its room temperature motional and orientational features in phospholipid bilayers must be known accurately to avoid any misinterpretation in using it in these types of studies. The aim of the present work was to reinvestigate the orientation distribution and motion characteristics of 16-DSA spin label in DPPC multibilayers. The orientation of the sample with respect to external magnetic field direction was monitored by a goniometer and ESR spectra in a plane perpendicular to the plane of glass slide were recorded with an angular increment of 5°. These spectra were used to study the variations of line separations, g factor, line widths and line amplitudes with orientation angle. Assuming a Gaussian distribution of the labels in the bilayers and attributing a restricted random walk motion to labels, we have simulated experimental spectra recorded for parallel and perpendicular orientations and have calculated the values of the distribution width (σ), tilt angle (δ) and random walk half amplitude (γ) of 16-DSA spin label in DPPC multibilayers.

1. Introduction

The electron spin resonance of nitroxide spin labels have provided novel information about structural and functional properties of biological and model membranes [1, 2]. The

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organization of these membranes can often be best characterized by studying macroscopically oriented samples. Membranes contain fluid bilayer regions in which the lipids tend to be oriented normal to the plane of the membrane [3,4]. Ordered fluid regions and its degree of order can be easily characterized directly from ESR study of spin labels inserted in the oriented membrane preparations. A Gaussian distribution of the spin labels incorporated in the membranes has been accepted in the literature to account for the ESR spectra obtained from model and biological membranes [3, 5, 6]. In the present work, we have used 16-doxyl stearic acid spin label (16-DSA) to study motional and structural properties of hydrophobic core of dipalmitoylphosphatidylcholine (DPPC) multibilayers by ESR spectroscopy. The distribution model of spin labels in the bilayers is combined with the models of anisotropic motion described in the literature [7, 8] to simulate recorded ESR spectra. The physical meaning of calculated distribution and motional parameters has also been discussed.

2. Materials and Methods

Chemicals

1,2-Dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) was purchased from Sigma (St. Louis, MO, USA) and used without any further purification. The stearic acid spin label 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxy (16-DSA) was obtained from Aldrich (Milwaukee, WI, USA). All other chemicals used in sample preparation were of reagent grade from Merck (Darmstadt, Germany). Distilled water was used throughout.

Preparation of planar DPPC samples

The procedure followed was essentially that of Libertini et al.,[5]. A solution of phospholipid and spin label (16-DSA) made by adding 10 μl of 10^{-2} M solution of 16-DSA to 10 mg of phospholipid in a flat flask was evaporated with the aid of a stream of nitrogen, followed by exposure to high vacuum for 2 h. After adding 2 ml of distilled water, on top of the thin film formed at the bottom of flask, the mixture was, first, agitated mechanically for 5 minutes, then sonicated for 60 sec. A small amount of the opalescent spin label containing DPPC dispersion was evaporated on a $20 \times 10 \times 1$ mm Corning glass cover slide kept at 39°C on a warming table for 30 minutes to evaporate all water in the film. The later was trimmed with a razor bland prior to performing any ESR measurements to avoid any contribution from the ridge surrounding it. Care was taken that spin label concentration was low enough to avoid any spin-spin interaction.

ESR measurements

All ESR spectra were recorded using a Varian E-L9" spectrometer with a TE_{104} double rectangular cavity. A home made goniometer was employed to monitor the orientation of lipid film with respect to external magnetic field in the microwave cavity. Accuracy

achievable with this goniometer was $\pm 1^\circ$. The majority of ESR spectra were recorded at room temperature (20°C) in a plane containing the normal of the lipid film with an angular increment of 5°. Orientation of the film was defined as the angle between the normal of the lipid film and external magnetic field. When the magnetic field was perpendicular to the plane of the film, it was specified as the $\theta = 0^\circ$ orientation. Spectra were recorded well below saturation at a microwave power level of 10 mW. 100 kHz field modulation frequency for phase sensitive detection and 0.1 mT peak to peak amplitude of the magnetic field modulation signal were used. Experiments were repeated with three different samples and it was concluded that measurements were perfectly reproducible.

Theoretical Background

Macroscopically oriented membrane preparations contain fluid bilayer regions in which the lipids tend to be oriented normal to the plane of the membrane. The degree of order in these regions can be directly determined from the ESR spectra of spin labels incorporated into the lipid bilayers. A Gaussian distribution model of orientations have been frequently used in the literature to account for the ESR spectra of spin labels ordered in model and biological membranes [3, 5, 6, 9]. This orientation model is combined with the models of anisotropic motion of spin label [7] to simulate experimental spectra recorded for planar DPPC films labeled with 16-DSA.

In lipid bilayers, a stearic acid spin label molecule rotates very rapidly around its long axis (z) which, in turn, makes a wobbling motion staying in a cone of tip angle γ [3, 6]. In the case of 16-DSA, this corresponds to wobbling of the nitroxide z axis, and thus the motion-average hyperfine parameters become

$$A_{\parallel} = \frac{1}{2}(A_{xx} + A_{yy}) + [A_{zz} - \frac{1}{2}(A_{xx} + A_{yy})]W \tag{1}$$

$$A_{\perp} = \frac{1}{2}(A_{xx} + A_{yy}) + [A_{zz} - \frac{1}{2}(A_{xx} + A_{yy})]\frac{1}{2}(1 - W), \tag{2}$$

where A_{xx}, A_{yy} and A_{zz} are the principal elements of the hyperfine tensor, and W is a function of the half amplitude γ of the wobbling motion and is defined as $W = (1 + \cos\gamma + \cos^2\gamma)/3$. Motion-averaged spectroscopic splitting factors ($g_{\parallel}$ and $g_{\perp}$) of wobbling molecules can also be expressed in terms of the g tensor principal elements. To do that one must replace the A' s with g' s in the last equations.

The orientation of a spin label molecule in a planar membrane can be characterized by the angle between the normal of the membrane and the long axis of the molecule (z-axis). The fraction of spin label molecules with z axis pointing within the zone bounded by θ_1 and $\theta_1 + d\theta_1$ on the surface of a unit sphere can be described by an orientation distribution function $P(\theta_1)$ as $P(\theta_1)d\theta_1$. A physically acceptable model is to assume that a Gaussian distribution of orientations exists in a cross-section of the unit sphere through the normal of planar membrane. Thus, for this model, the orientation distribution function $P(\theta_1)$ can be taken as [6]:

$$P(\theta_1) = N^{-1} \exp[-(\theta_1 - \delta)^2/(2\sigma^2)] \sin\theta_1, \quad (3)$$

where N is the normalization constant, σ is the orientation distribution width parameter and δ is the angle of tilt. In this expression $\sin\theta_1$ results from the area element in spherical coordinates.

3. Results

Planar sample prepared on a Corning glass slide was located inside the microwave cavity using a home-made goniometer so that the sample could be rotated around an axis staying in the plane of sample. Orientation angle of the sample was measured as the angle between the normal to the sample plane and the external magnetic field, and it was taken to be zero when the external magnetic field was parallel to the normal of the sample plane. Three different spectra recorded at three different orientations are given in Figure 1. As it can be seen from this figure, the spectra consist of three lines for all orientations and their characteristic features depend strongly on the orientation of external magnetic field. Separations between low field and high field lines were the largest and the narrowest when the orientation angle was 0° and 90°, respectively. The variations of the low-center and center-high line separations or hyperfine splitting in a plane containing the sample normal is shown in Figure 2. Both line separations had a magnitude of 0.18 mT when θ was 0° and they decreased with increasing orientation angle. The rate of decrease was higher for center-high line separation than that of low-center line separation. Those separations were found to follow two different theoretical sinusoidal curves as indicated in Figure 2 by solid-lines.

We have also studied the variation of spectroscopic splitting factor g with orientation angle. The results of this study is given in Figure 3. The g-factor was observed to vary in an interval of 2.0043-2.0060 which is well within the experimental error limits (± 0.0004) for the determination of this factor. The g factor increased with increasing orientation angle, that is, upon increasing θ the whole spectrum moved toward low magnetic fields. This result is consistent with the fact that when magnetic field is parallel to the long axis of 16-DSA $\theta = 0^\circ$ the g value is smaller than the value obtained for perpendicular orientation ($\theta = 90^\circ$). Calculated g values for $\theta = 0^\circ$ and $\theta = 90^\circ$ orientations were not very far from the values obtained for fatty acid spin labels immobilized on lyophilized bovine serum albumin [3, 9]. As in the case of line separation, the variation of g values with orientation angle follows a sine curve of the type $g^2 = g_\perp^2 \sin^2\theta + g_\parallel^2 \cos^2\theta$. The solid curve given in Figure 3 shows this variation.

The narrow lines resulting from 16-DSA indicate a motion which is very rapid compared to the other spin labels studied. The line widths of these lines vary appreciably with orientation of sample except the width of the center line (Figure 4). The latter changes slightly over $0^\circ - 90^\circ$ orientation angles. The widths of high and low lines experience increases of nearly 50 % when orientation angle θ is changed from 0° to 90°, but it is difficult to say that these variations follow well defined theoretical curves as in the line separation and g-factor cases. This is partly due to the fact that g-factors and line

separations of 16-DSA spin labels in DPPC sample show angular distribution, that is, measured line widths are not the widths originating directly from spin label oriented in unique way inside the sample. The high line width values calculated for $\theta = 0°$ (parallel) orientation can be seen as a natural result of the relatively high rate of change of the g factor and line separation curves. Line amplitudes also vary with the orientation of sample. As seen in Figure 5, a decrease in the line width bring about an increase in the line amplitudes and these changes follow nearly sine curves.

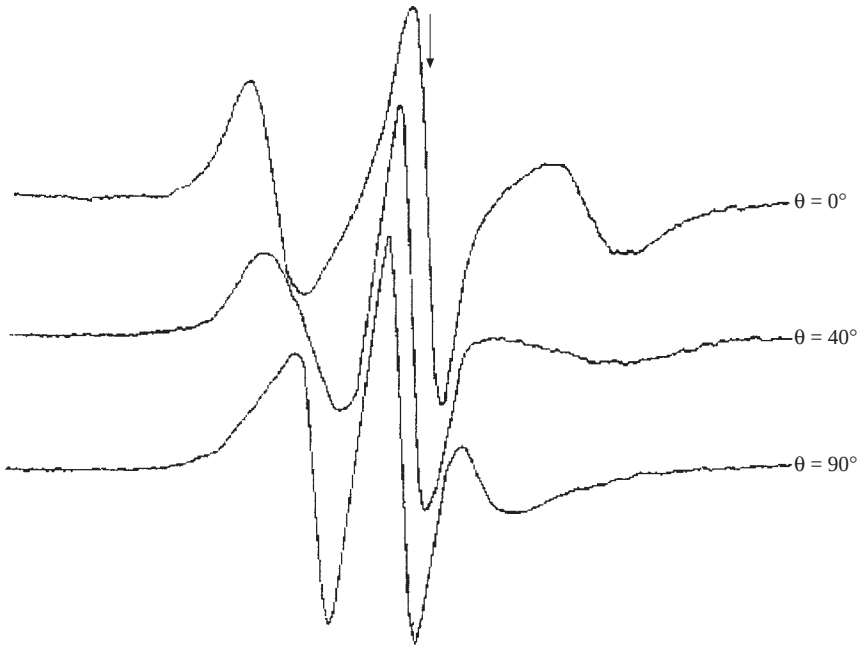

Figure 1. Experimental spectra recorded for different orientations. Arrow indicates the DPPH Signal center

To test the effects of different parameters on the features of ESR spectrum of 16-DSA spin label in DPPC sample we have calculated the distribution of spin labels and corresponding spectra for various sets of parameters. For convenience, the number of 16-DSA spin labels included in the calculation was limited to 2000. With the aid of a computer program designed to generate random number, all 2000 DSA's were assigned random orientations. An ESR spectrum of each spin label was calculated from equations 1,2 and 3, assuming that each individual first-derivative ESR line had a Gaussian line shape and was 0.15 mT wide ($\Delta H_{pp} = 0.15$ mT). Before summing these spectra directly, the amplitude of each spectrum was weighed by the orientation distribution function given in Eq.3 taking the mean tilt angle (δ) zero. Then, the resulting 2000 spectrum were summed to get each final compound spectrum. The results are given in Figure 6. For small value of γ, the amplitude of wobbling motion is small and thus the orientational

distribution of spin labels is the only major parameter affecting the deviation of spectra from that of a relatively ordered sample. If however the orientation distribution parameter σ is also small, then the ESR spectra approaches to the spectra of a highly ordered and doped sample with 16-DSA spin labels. Increases in γ and σ parameter values bring about increases in the motional and spatial disorders which give rise to liquid-like ESR spectra as seen in the bottom row of Figure 6. This computer generated spectra illustrates the degree of order as a function of both orientation distribution and random walk amplitude parameters (σ, γ).

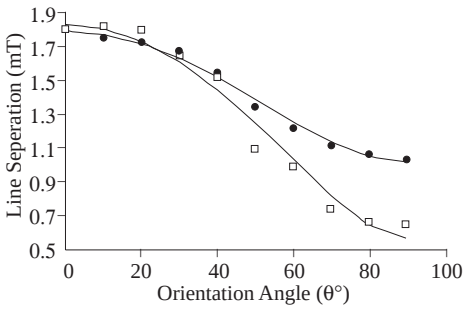

Figure 2. Variations of line separations with orientation angle. (•) denotes low-center line; (□) center-high line; (-) theoretical

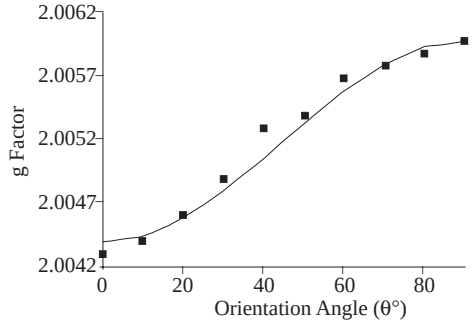

Figure 3. Variations of g-factor with orientation angle. (■) denotes experimental; (-) theoretical

Experimental spectra recorded at parallel and perpendicular orientations were simulated based on the above model by means of a computer program constituted specially for this purpose. The best values of the spectral parameters were determined by varying σ, δ and γ parameters and the resonance line-shape parameters until the difference between observed and calculated spectra were minimized. The independent variables for each simulation include the distribution width σ, tilt angle δ, the width and shape of individual ESR lines which are assumed to be Gaussian. The principal values of the hyperfine tensor (A_{xx}, A_{yy}, A_{zz}) and of the g-value tensor (g_{xx}, g_{yy}, g_{zz}) were kept constant and taken as those given in the literature for this label molecule [3, 9]. The effects of molecular motion were incorporated into the model by replacing the principal values of A and g by their motion-averaged counterparts using equations 1 and 2. The weighted spectra from all orientations were summed to get the total spectrum. Calculated spectra with this procedure and corresponding experimental spectra are given in Figure 7. As it is seen, the agreement between experimental and calculated spectra is good but not perfect. We suspect that a principal source of the remaining discrepancy arises from line broadening due to defective areas in the film, since trimming the edges of the film increased the value of $\Delta A = A_{\parallel} - A_{\perp}$ as much as 0.2-0.3 mT. The values of the parameters representing orientational and motional disorders ($\sigma = 20°$, $\delta = 35°$ and $\gamma = 50°$, respectively) were found to be relatively high. This indicates that even at room temperature, both factors

contribute appreciably to the shape of ESR spectra of the planar DPPC sample prepared in the present work.

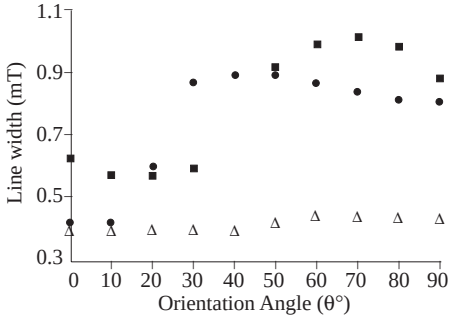

Figure 4. Variations of line with orientation angle. (•) denotes low line; (Δ) center line; (■) high line

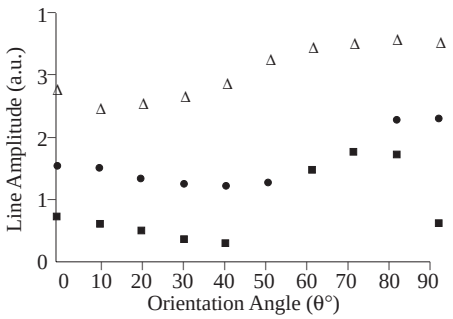

Figure 5. Variations of line amplitudes with orientation angle. (•) denotes low line; (Δ) center line; (■) high line

4. Discussion

It is immediately apparent from Figure 1 that the features of the ESR spectra change as the planar sample of DPPC is rotated in the magnetic field. This means that 16-DSA spin labels are not randomly oriented in the DPPC sample. As is known the spectra of dilute concentration of fatty acid spin labels in ordered diamagnetic media consist of three sharp lines and the anisotropy is reflected in the splitting between the resonance lines and the absolute position of the center of the spectrum. The principal values of the hyperfine splitting and g tensors of 16-DSA in ordered media are reported to be; $A_{xx} = 0.63$ mT, $A_{yy} = 0.58$ mT, $A_{zz} = 3.36$ mT and $g_{xx} = 2.0088$, $g_{yy} = 2.0061$, $g_{zz} = 2.0027$ [3, 9]. This shows that the largest splitting occurs when the magnetic field is parallel to the spin label long molecular axis z, and the minimum splitting occurs with the magnetic field is perpendicular to this axis. When these data are compared to those of Figure 1, it is clear that the z axis of 16-DSA molecule exhibits a broad Gaussian distribution of orientations throughout the multibilayers and /or the molecule undergoes rapid rotational motions about the z axis. Temperature studies have shown that, in fact, considerable molecular motion of nitroxide occur in lipid samples [2,10-16]. The molecules rotate about their long molecular axis. This motion is more restricted in regions closer to the polar part of the bilayer, the freedom increasing in region closer to the hydrophobic end of the molecules as in the case of 16-DSA spin label. The molecular long axis can also perform a restricted random walk within a cone whose axis is parallel to the normal of the bilayer. The intrachain motion due to trans-gauch isomerization and flip-flop motions can also occur. All these motions and orientation distribution of spin labels contribute considerably to the feature of ESR spectra.

Motion about long molecular axis z of the stearic acid spin labels may be rapid

($gg10^8s^{-1}$) or slow ($ll10^8s^{-1}$) in the lipid samples. 16-DSA spin label exhibits rapid rotation about its long axis in DPPC planar lipid samples which serves to average the x and y components of the **(g)** and hyperfine splitting **(A)** tensors. In every case studied thus far it has been found that the principal axis of **g** and **A** tensors coincide [6]. The splitting or line separation for 16-DSA in the DPPC multibilayers differ slightly from preparation to preparation, and is correlated with the macroscopic appearance of the film. Obvious defective regions in the other wise translucent glassy films are nearly always associated with a somewhat lower difference between the splittings of the two right-angle orientations.

In Figure 2, the experimental anisotropy data are plotted against the angle θ. At $\theta = 0°$, that is, when external magnetic field was parallel to the normal of the slide, spectra were recorded every 5° between 0° and 90° as the slide was rotated in the magnetic field. In the ideal situation, namely, in the case where all 16-DSA spin labels are aligned with their long axis exactly perpendicular to the glass slide the difference $\Delta A = A_{\parallel} - A_{\perp}$ is simply $A_{zz} - (A_{xx} + A_{yy})/2 = 2.75$ mT for 16-DSA. The observed ΔA for the same label clearly falls well below this maximum value and it varies between 0.75-1.15 mT. The line separation or hyperfine splitting with magnetic field perpendicular to the sample normal ($\theta = 90°$) is less accurate than in the complementary case because of the broad asymmetries present in the spectra. However, anisotropic motion in the form of a restricted rapid random walk and the distribution of orientations must also be considered if line splittings and line positions are to be evaluated.

Deviations of hyperfine and g-values from those expected for perfect alignment were accounted for using a model in which the spin-label long axis executes a restricted rapid random walk within a cone of half angle γ whose axis is perpendicular to the bilayer surface. The rapid conical motion averages the x- and y- component of the g and hyperfine splitting tensors. When the rate of this motion becomes comparable to the energy difference between the various g and hyperfine states appreciable line broadening effects are expected. As is seen from Figure 1; the high line is the most anisotropic as a result of the combined effects of hyperfine splitting and g-value anisotropies. Such an effect is quite common for nitroxides in isotropic solution when the molecular motion is slowed down by viscosity or other effects.

The projection of the 16-DSA spin label molecules long axis in one-half of the DPPC bilayer on a plane perpendicular to the slide plane is given at the top of Figure 6. This computer generated projection illustrates the degree of order as a function of the orientation distribution width parameter σ. As seen from this figure, small values of σ correspond to a very high degree of spatial order. The degree of order decreases rapidly with increasing σ. However, the difference in appearance decreases for high σ. In the limit as $\sigma \to \infty$, the spin labels are randomly ordered in the sample, but for practical purposes the orientation can be considered random well before $\sigma \to \infty$.

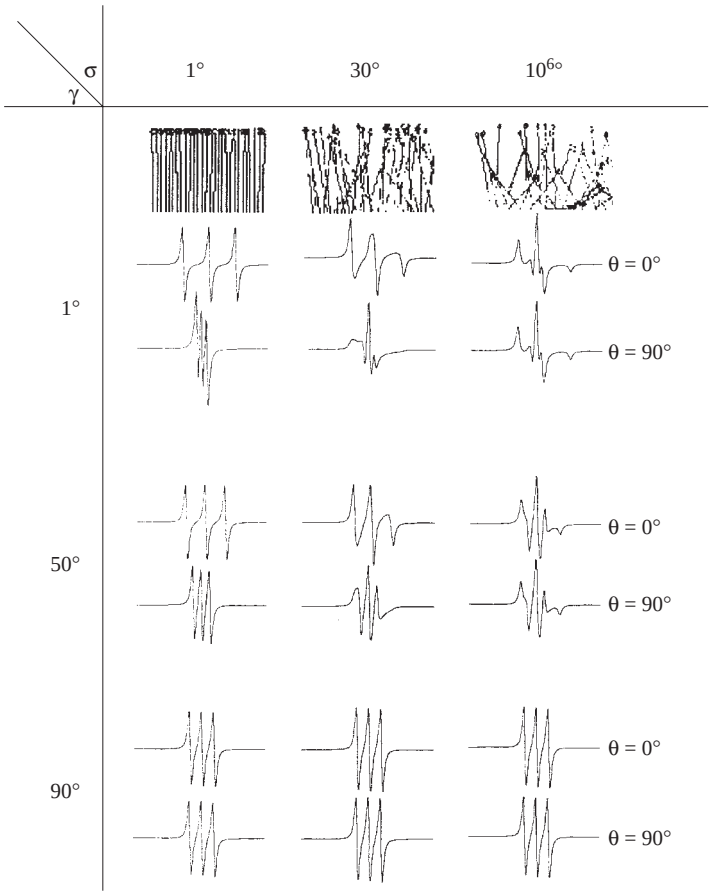

Figure 6. Projection of spin label z-axis on a plane perpendicular to the glass slide and computer generated ESR spectra for different values of Gausian distribution width σ and random walk half γ. In calculating ESR spectra, width of each resonance line and tilt angle of distribution were taken as 0.15 mT and zero, respectively

In Figure 6, the ESR spectra of 16-DSA spin label for different values of restricted random walk half amplitude γ and orientation distribution with σ parameters are also given. From this figure it is clear that for small values of σ and γ, ESR spectra do in fact resemble experimental single crystal spectra. Keeping γ small but increasing σ or vice versa gives rise to the familiar rigid glass spectrum of a randomly oriented sample. In conclusion, restricted random walk half amplitude γ and orientation distribution width parameter σ play an important role in determining the shape of ESR spectrum of 16-DSA incorporated in DPPC planar samples.

The data derived from simulation of experimental spectra (Figure 7) substantiate the assumption of rapid or intermediate reorientation rates of spin label molecules and their

orientation distribution in DPPC multibilayers. Therefore, the model based on a distribution of orientation about a direction normal to the glass slide and rapid random walk appears to account for the prominent feature of the ESR data. In summary, intercalating of 16-DSA spin labels into DPPC multibilayers provides a powerful approach in obtaining information about the extent of orientation and motional freedom of the lipid components in the hydrophobic core of the bilayers.

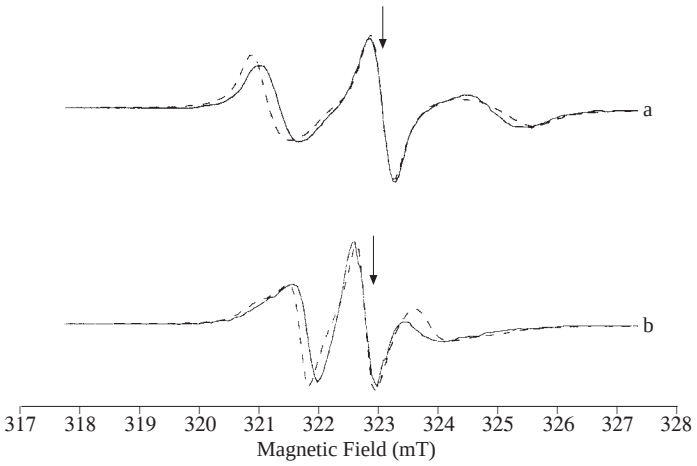

Figure 7. Room temperature experimental and simulated spectra for, a-parallel ($\theta = 0^\circ$) and b- perpendicular ($\theta = 90^\circ$) orientations. Parameter values giving best simulation were: $\gamma = 50^\circ$, $\sigma = 20^\circ$ and $\delta = 35^\circ$. Arrows indicate the center of DPPH signals

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