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Spin-Label and Deuterium Order Parameter Discrepancies in Bilayers: One Possible Explanation†

Ronald P. Mason* and Carl F. Polnaszek‡

ABSTRACT: We have simulated electron spin resonance spectra of anisotropically immobilized spin labels of the type seen in lipid and soap-like bilayers using a rigorous formalism which explicitly includes the effects of spin-label motion. In most bilayer systems, spin-label experiments have shown lower order parameters then deuterium-label experiments. In the past this apparent decrease in the order parameters was thought to reflect the distortion of the bilayer by the doxyl ring of the spin probes. We wish to report that this type of discrepancy may be due to the neglect of important motional effects in the time-independent effective Hamiltonian formalisms used in previous interpretations of anisotropically immobilized spin label spectra. That the true order parameters may be the same can be shown by including slow motional corrections in the effective Hamiltonian formalism. The larger volume of the doxyl ring may change the apparent order parameter by increasing the importance of the slow motional effects, as opposed to causing a real decrease in the order parameter, as previously proposed.

Seelig & Niederberger (1974), Seelig & Seelig (1974), and Stockton et al. (1976) have found that bilayers have differences in the order parameter, S, as determined from deuterium magnetic resonance (^{2}H NMR) experiments with selectively deuterated hydrocarbon chains, as opposed to S as determined from spin-labeling experiments. They found that S determined by deuterium labeling, S^d, is generally greater than S determined from spin labeling, S^s. An example of the published data is shown in Table I. The stearic acid spin probes are thought to introduce distortion of the bilayer at the site of the doxyl ring, and to reflect the response of the bilayer to the probe perturbation. The nitroxide disruption of the bilayer is reflected in this view by a generally lower order parameter resulting in $S^s < S^d$ (Seelig & Niederberger, 1974; Stockton et al., 1976; Seelig, 1977).

McFarland & McConnell (1971) have proposed that a cooperative tilt of the phospholipid chain near the polar head group could account for the flexibility gradient observed in spin-labeling experiments. They have also noted that, if the lifetime of the statistically tilted chain regions were short on the deuterium resonance time scale (10^{-5} to 10^{-6} s) but long on the ESR time scale (10^{-7} to 10^{-8} s), S^d would show a lower gradient of order than S^s (Gaffney & McConnell, 1974). Seelig & Seelig (1974) have observed that in this case S^s would be greater than S^d. The egg yolk lecithin bilayer appears to be an example where a time-dependent tilting of the hydrocarbon chain is responsible for $S^s > S^d$ (Gaffney & McConnell, 1974; McConnell, 1976; Seelig & Seelig, 1977).

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† From the Veterans Administration Hospital, Minneapolis, Minnesota 55417, and the Departments of Laboratory Medicine and Pathology, and Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received August 17, 1977.*

* Address correspondence to this author at: Laboratory of Environmental Biophysics, NIEHS, P. O. Box 12233, Research Triangle Park, N.C. 27709.

‡ Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A OR6. This paper is NRCC Publication No. 16589.

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We wish to indicate how motional corrections to the S formalism, as applied to fatty acid and phospholipid spin probes, may be responsible for S^s appearing to be less than S^d, as is more commonly the case. In nearly all of the theoretical analyses of the fatty acid spin-label spectra (Schindler & Seelig, 1973; Gaffney & McConnell, 1974; Van et al., 1974, and references therein), McConnell (1976) has emphasized that the primary assumption is that the exact, time-dependent spin Hamiltonian for this problem, $\mathcal{H}(t)$, can be decomposed into two parts. The first is a time-averaged effective Hamiltonian, $\mathcal{H}'$, which determines the spectral line positions. The second part is a time-dependent perturbation, $(\mathcal{H}(t) - \mathcal{H}')$, which is characterized by short rotational correlation times and only affects line widths.

$$\mathcal{H}(t) = \mathcal{H}' + (\mathcal{H}(t) - \mathcal{H}')$$

The use of such an effective Hamiltonian is based on two assumptions. The first assumption is that the motion about the symmetry axis of $\mathcal{H}'$ is fast on the ESR time scale and that any residual time-dependence does not effect spectral line positions (Hubbell & McConnell, 1969; McConnell & McFarland, 1970). This motion may be described by an effective rotational diffusion rate, $R_\parallel$, where the rotational correlation time about the motional symmetry axis, $\tau_{R_\parallel}$, is equal to $1/(6R_\parallel)$. In the absence of a tilt angle, this axis is coincident with the normal to the bilayer, and does not generally coincide with a nitroxide molecular axis, such as the 2 p–π orbital of the nitrogen atom. The second assumption made in the effective Hamiltonian formalism is that motion perpendicular to the $\mathcal{H}'$ symmetry axis, characterized by the rate $R_\perp$, is slow on the ESR time scale.

Mason et al. (1974) have examined the results of the breakdown of the assumption that $\tau_{R_\parallel}$ is fast on the ESR time scale. When the rotational correlation time about the motional symmetry axis, $\tau_{R_\parallel}$, is longer than 2×10^{-10} s, and the true S is equal to 0.34, S^s was found to increase monotonically with longer $\tau_{R_\parallel}$'s to a value of 0.53 at $\tau_{R_\parallel} = 1.0 \times 10^{-9}$ s. At $\tau_{R_\parallel} = 6.0 \times 10^{-9}$ s the inner hyperfine extrema, which define the apparent $\mathbf{T}_\perp$'s, are no longer resolved (Mason et al., 1974). Only at this point do the spectra no longer reflect apparent cylindrical symmetry of the magnetic parameters. As this example indicates, any breakdown of the assumption that $(\mathcal{H}(t) - \mathcal{H}')$ is completely averaged by short correlation times can only lead to S^s greater than the true S.

We will now examine the consequences of a breakdown of the second assumption. This assumption, that part of the exact, time-dependent spin Hamiltonian is in fact time independent, would appear to be difficult to justify in a bilayer near room temperature, because this assumption corresponds to assuming that motion transverse to the above mentioned motional symmetry axis, $R_\perp$, is very slow on the ESR time scale (Mason et al., 1974).[1] If the motional symmetry axis is coincident with the straight rigid stick conformation of the all-trans polymethylene backbone, then $\tau_{R_\perp}$ less than 3×10^{-7} s, where $\tau_{R_\perp} = 1/(6R_\perp)$, will decrease S^s below $S = 1.0$. More explicitly, $\mathbf{T}_\parallel'$ will be less than $\mathbf{T}_z$ (Goldman et al., 1972), where $S = (\mathbf{T}_\parallel' - \mathbf{T}_\perp')/(\mathbf{T}_z - \mathbf{T}_x)$ (Seelig, 1970; Hubbell & McConnell, 1971). If S is less than 1.0, then even slower motions will result in a decrease in S^s. If both the first and second assumptions are fulfilled, then the axis of $\tau_{R_\parallel}$ will be coincident with the axes of $\mathbf{T}_\parallel'$ and $\mathbf{g}_\parallel'$ and the axis of $\tau_{R_\perp}$ will be coincident with the axes of $\mathbf{T}_\perp'$ and $\mathbf{g}_\perp'$.

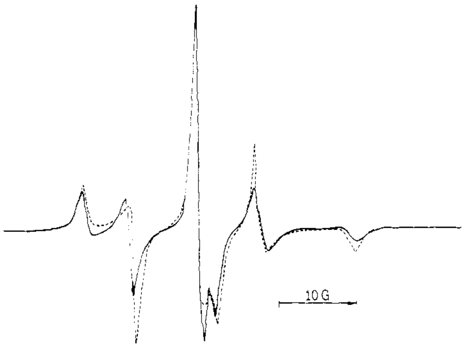

FIGURE 1: A comparison of computed anisotropically immobilized spectra. (a) $S = 0.50$ and $\tau_{R_\perp} = \infty$, where a time-independent effective Hamiltonian has been assumed (- - -). (b) $S = 0.57$ and $\tau_{R_\perp} = 6.2 \times 10^{-8}$ s, where no part of the Hamiltonian is time-independent (—). When these simulations are analyzed in the manner of experimental spectra (Hubbell & McConnell, 1971), S^s is found to be equal to 0.50 in both cases. Spectra of spin probes in macroscopically disordered bilayers are independent of the tilt parameter, which is the angle between the axis of $\mathbf{T}_\parallel'$ and the normal to the bilayer plane.

Hubbell & McConnell (1971) noted that a rapid rocking of the rigid stick axis about the normal to the bilayer surface would produce a reduction of the order parameter over and above that due to gauche–trans isomerizations. This rapid averaging is conceptually similar to the restricted random walk of the long probe axis within a cone defined by the angle γ (Jost et al., 1971; Smith, 1971; Lapper et al., 1972; Van et al., 1974). Motion of the rigid stick or of the cone which is not very slow on the ESR time scale corresponds to a breakdown in the assumption that spectral line positions are determined solely by a time-independent effective Hamiltonian. Although the fatty acid spin labels are not expected to tumble end-over-end in an ordered bilayer, there may be appreciable motion with the entire lipid moving as one entity, such as a wagging over fairly large angles. This time dependent tilting will behave as an effective $\tau_{R_\perp}$ in the analysis. The primary effect of this overall motion (10^{-7} to 10^{-8} s) is to reduce the observed order parameter below the true order parameter.

This kind of effect could account for the observation where S^s is less than S^d. In Table I we show S^s and S^d determined under identical conditions along with the calculated value of $\tau_{R_\perp}$ necessary to reduce S^d to the S^s value. All the $\tau_{R_\perp}$'s are fairly long and are typical of small, spherical spin labels in viscous organic glasses (Hwang et al., 1975). In Figure 1 we show computed spectra of an anisotropically immobilized fatty acid spin label which are similar to the observed spectrum seen in the macroscopically disordered decanol–sodium decanoate bilayers (Figure 4 of Schindler & Seelig, 1973). These spectra show that the effect of a long, but finite, $\tau_{R_\perp}$ is all but indistinguishable from a decrease in S^s.

In Table I we have tabulated the values of $\tau_{R_\perp}$ that decrease the S^d order parameters of Seelig & Niederberger (1974) to their observed S^s values. As illustrated in Table I, the highly anisotropic immobilization of spin probes in lipid bilayers is consistent with the effective Hamiltonian being characterized by correlation times as short as 10^{-8} s. In such cases the value of $\tau_{R_\perp}$ is a significant factor in determining the positions of the inner and outer hyper fine extrema used in the experimental determination of S^s.

The existence of a measurable tilt between the axis of $\mathbf{T}_\parallel'$ and the normal to the bilayer plane in egg-yolk lecithin has been presented as evidence that the time-independent part of

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[1] One of the reviewers noted that ^{2}H NMR results from bilayers of deuterated lipids indicate that $\tau_{R_\perp}$ is longer than 10^{-4} s (see Stockton et al., 1976), but that spin-labeled lipids may have shorter $\tau_{R_\perp}$'s as a result of the distortion of the bilayer induced by the doxyl ring.

TABLE I: The Effect of Slow Motion on Spin-Label Order Parameters.

Labeled carbon atom no.	Deuterium label order parameter S^d	Spin-label order parameter S^s	Calcd $\tau_{R_\perp}$ (ns) that will decrease S^d to S^s
6	0.572[a]	0.50[a,b]	62.0[c]
7	0.576	0.43	18.6
8	0.480	0.35	21.6
9	0.378	0.25	20.8

[a] Data from Seelig & Niederberger (1974) for the sodium decanoate (32 wt %), decanol (38 wt %) and water (30 wt %) bilayer system. S^d is within 0.03 of S^s for carbon atoms 4, 5, and 10. [b] Pseudo-axial rigid limit spectra were calculated from the axially symmetric magnetic parameters ($\mathbf{T}_\perp'$, $\mathbf{T}_\parallel'$, $\mathbf{g}_\perp'$, and $\mathbf{g}_\parallel'$), which were determined from S^s, where $S^s = (\mathbf{T}_\parallel' - \mathbf{T}_\perp')/(\mathbf{T}_z - \mathbf{T}_x) = (\mathbf{g}_\parallel' - \mathbf{g}_\perp')/(\mathbf{g}_z - \mathbf{g}_x)$, $\mathbf{T}_x = \mathbf{T}_y = 6.15$ G, $\mathbf{T}_z = 33.00$ G, $\mathbf{g}_x = \mathbf{g}_y = 2.0074$, and $\mathbf{g}_z = 2.0027$. The **g** and **T** tensors in the molecular principal axis system were taken from Schindler & Seelig (1973), but we have assumed $\mathbf{T}_x = \mathbf{T}_y$ and $\mathbf{g}_x = \mathbf{g}_y$, because we were not making direct comparisons with experimental spectra. [c] The rotational correlation time transverse to the motional symmetry axis necessary to cause the superposition of the inner and outer hyperfine extrema of the ESR spectrum calculated with $S = S^d$ and with the $S = S^s$ and $\tau_{R_\perp} = \infty$ spectrum. Motionally dependent spectra with a peak-to-trough Lorentzian residual line width of 0.3 G had comparable total line widths to time-independent spectra with residual line widths of 1.0 G (see Figure 1).

the Hamiltonian is characterized by lifetimes of at least 10^{-7} s (McConnell, 1976). This tilt has only been observed near the polar head group of egg-yolk lecithin where $S^s > 0.7$ and $S^s > S^d$. Whereas in the reported data where S^s is less than S^d, S^s is less than 0.7. In these cases the increased randomness of the hydrocarbon chains is correspondingly higher and all of their motions are intuitively more rapid.

Besides decreasing the apparent S^s value, $\tau_{R_\perp}$'s less than 3×10^{-7} s will preferentially broaden the outer hyperfine extrema (Mason et al., 1974, 1977). This effect of finite $\tau_{R_\perp}$'s is similar to that seen with an orientation-dependent inhomogeneous line width in rigid limit (Hwang et al., 1975; Mason et al., 1977) or pseudo-axial rigid limit simulations (McFarland & McConnell, 1971; Schindler & Seelig, 1973; Gaffney & McConnell, 1974) of experimental spectra. Environmental heterogeneity in the vicinity of a nitroxide has clearly been demonstrated by resolving two $\mathbf{T}_z$'s in an ethanol glass (Hwang et al., 1975) and two $\mathbf{T}_\parallel$'s in an egg lecithin bilayer (Sanson et al., 1976). If the heterogeneity is too small to resolve, then preferential broadening of the outer hyperfine extrema could be expected and is involved as a rationale for the need to use an orientation-dependent residual line width. However, the preferential broadening of the outer hyperfine extrema could also be the result of finite $\tau_{R_\perp}$'s (Mason et al., 1974). In fact, Mason et al. (1977) recently found in erythrocyte membranes that decreases in widths of outer hyperfine extrema correlated with increases in $\mathbf{T}_\parallel'$ as is consistent with $\tau_{R_\perp}$'s on the order of 10^{-7} to 10^{-8} s. In addition, in liquid crystal systems Polnaszek & Freed (1975) have found slow motions characterized by times of 10^{-7} to 10^{-8} s which similarly effect the widths and positions of the outer hyperfine lines of spin probe spectra.

Although the ^{2}H NMR results clearly indicate that $\tau_{R_\perp}$ is longer than 10^{-4} s in bilayers containing deuterated lipids (Stockton et al., 1976), shorter $\tau_{R_\perp}$'s in bilayers containing doxyl spin labels are possible as a result of the distortion induced by the doxyl ring. Our results are consistent with a shorter $\tau_{R_\perp}$ being the cause of the apparent decrease in S^s relative to S^d. Our interpretation differs from earlier discussions where disruption of the bilayer by the doxyl ring was thought to cause a real decrease in S^s.

Mason et al. (1974, 1977) have noted that S^s may be larger (smaller) than true S as a result of $\tau_{R_\parallel}(\tau_{R_\perp})$ being longer (shorter) than assumed in the effective Hamiltonian formalism (Hubbell & McConnell, 1969, 1971; McConnell & McFarland, 1970). The explicit dependence of S^s on slow molecular motion can account for the observation of artifactual polarity changes (Mason et al., 1974), the correlation of the decrease in the widths of the outer hyperfine extrema with the simultaneous increase in their separation ($2\mathbf{T}_\parallel'$) (Mason et al., 1977), and possibly explain many of the observed discrepancies between S as determined from ESR and ^{2}H NMR experiments, where $S^s < S^d$. One motion which is consistent with finite $\tau_{R_\perp}$'s would be the time-dependent tilting of the hydrocarbon chains on the time scale of 10^{-7} to 10^{-8} s. This type of motion on the time scale of 10^{-6} to 10^{-7} s has already been postulated by Gaffney & McConnell (1974) to explain the observation of $S^s > S^d$ near the polar head group of egg-yolk lecithin.

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