More on the Motional State of Lipid Bilayer Membranes: Interpretation of Order Parameters Obtained from Nuclear Magnetic Resonance Experiments†

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ABSTRACT: Proton and deuterium order parameters measured for the liquid crystalline phase of unsonicated lipid bilayer membranes are interpreted in terms of two motions: (i) chain reorientation and (ii) chain isomerization via kink diffusion. The observed order parameters are found to be compatible with angular deflections of the chain of about 50° with respect to the bilayer normal, coupled with a probability of trans orientation of a methylene segment in the upper part of the chain of about 0.8–0.9. The motional model can be shown to account for the dynamic properties of the membrane system as measured by nuclear magnetic relaxation measurements, assuming that the chain isomerization occurs at a rate of $\sim 10^{10}$ s^{-1} and chain reorientation at a rate of $\sim 10^{7}$ s^{-1}. Analysis of proton and deuterium line-width data in terms of this model shows that sonication has the effect of increasing the rate and amplitude of chain reorientation without substantially changing the isomerization motion along the acyl chain. These conclusions are briefly compared with similar observations recently reported in Raman spectroscopic studies.

The state of motion of lipid molecules in bilayer membranes can be ascertained in a number of ways. The physical methods, which have yielded the most detailed information, include electron spin resonance (ESR)[1] measurements on lipid spin labels (Hubbell and McConnell, 1971; Jost et al., 1971; Schindler and Seelig, 1974; Schreier-Muccillo et al., 1973), proton (^{1}H) and deuterium (^{2}H) nuclear magnetic resonance (NMR) (Seiter and Chan, 1973; Feigenson and Chan, 1974; Horwitz et al., 1972; Hemminga and Berendsen, 1972; Seelig and Seelig, 1974a,b; Stockton et al., 1976) and more recently Raman spectroscopy (Mendelsohn et al., 1976; Gaber and Peticolas, 1976). On the basis of these studies, it is now clear that the molecular motions in these systems are restricted. One consequence of this motional restriction, or molecular order, is the incomplete averaging of second-rank tensor interactions, which is revealed experimentally by: (i) nuclear dipolar broadening in the ^{1}H NMR spectra (Seiter and Chan, 1973; Hemminga and Berendsen, 1972), (ii) quadrupolar splittings in the ^{2}H NMR spectra (Seelig and Seelig, 1974a,b; Stockton et al., 1976), and (iii) effects in the ESR spectra arising from anisotropies in the electronic Zeeman and the nuclear hyperfine interactions (Hubbell and McConnell, 1971; Jost et al., 1971).

A quantitative measure of the molecular order is given by the order parameter. Indeed order parameters for lipid molecules in bilayer membranes have been determined using all of the above magnetic resonance methods. Typically the values of the order parameters determined by the various methods do not agree, which is particularly evident when the order parameters deduced from ESR experiments are compared with those deduced from deuterium quadrupolar splittings (Seelig and Seelig, 1974a; Seelig and Niederberger, 1974a). Even the trends are different, as the ESR measurements suggest a continuous flexibility gradient along the hydrocarbon chain of the lipid molecules, whereas no such gradient is observed in the ^{2}H NMR experiments for the upper two-thirds of the chain. These particular discrepancies have been attributed to effects arising from perturbations of the system caused by the rather large nitroxide spin label used in the ESR experiments. However, it should be noted that the time scale of the NMR experiment is much longer, and there is therefore no a priori reason to expect the two sets of order parameters to have the same values (Gaffney and McConnell, 1974). In fact, they would be different if the techniques sample the motions differently. Interpretation of the order parameters may therefore not be as straightforward as has been assumed.

The purpose of this communication is to elaborate on this very point and to attempt to relate the order parameters determined by the various techniques. As a result of this exercise, a model for the molecular motions which accounts for most of the available data can be presented. Finally, we shall show how some recent results on the effect of sonication on the structure of lipid bilayers also can be understood in terms of this motional model (Stockton et al., 1976; Mendelsohn et al., 1976; Gaber and Peticolas, 1976).

The Order Parameter

In motionally restricted systems it has been customary to describe the extent of order with respect to a laboratory frame of reference. In general, this requires specifying a matrix of order parameters. However, when the motions are such that the tensor interaction has *effective* axial symmetry, all the motional information can be referred to this axis and is expressed in terms of a single order parameter.

Consider the first-order nuclear dipolar interactions between two spins. It is well-known (Abragam, 1961) that this depends on the quantity $\overline{(3\cos^2\theta - 1)}$, where θ is the angle between the internuclear vector, $\vec{r}$, and the applied magnetic field, and the average is taken over the range of angles the vector $\vec{r}$ traverses on the time scale of observation. If this motion allows the

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[1] Abbreviations used: ESR, electron spin resonance; NMR, nuclear magnetic resonance.

Hamiltonian to retain axial symmetry relative to a laboratory set of axes, then

$$\overline{(3\cos^2\theta - 1)} = \frac{1}{2}\overline{(3\cos^2\beta - 1)}(3\cos^2\theta' - 1) \quad (1)$$

θ' is now the angle between the applied magnetic field and the effective laboratory fixed symmetry axis, $\hat{\mathbf{d}}$, usually referred to as the director. β is the angle between $\hat{\mathbf{r}}$ and $\hat{\mathbf{d}}$. The order parameter, S_β, is then defined by

$$S_\beta = \frac{1}{2}\overline{(3\cos^2\beta - 1)} \quad (2)$$

The effect of the above coordinate transformation is to reduce the nuclear dipolar interaction to a product of a scalar quantity, the order parameter, and a function describing the orientation of the director relative to the applied magnetic field. The order parameter reflects the motion of the vector $\hat{\mathbf{r}}$ about the director and is independent of the orientation of that director.

Measurements of the proton–proton dipolar interactions and deuterium quadrupolar interactions in oriented lipid bilayer membranes as a function of the orientation in the magnetic field (Hemminga and Berendsen, 1972; Seelig and Seelig, 1974b) show that, on the nuclear magnetic resonance time scale, effective axial symmetry exists about the bilayer normal. The question remains as to whether the order parameters need only be interpreted in terms of intramolecular motions (Seelig and Seelig, 1974; Stockton et al., 1976; Marčelja, 1974; Schindler and Seelig, 1975), or whether in fact rigid-body motions of the chains contribute to the reduction of the order parameter as well. The tendency in the past has been to dismiss the importance of the rigid-body type motions for lipid bilayers (Seelig and Seelig, 1974a). However, there is much evidence from studies of many liquid crystals that the molecules in these systems reorient about an average director (Doane, 1976). If similar reorientations of lipid molecules or the chains of the lipid molecules take place in the bilayer, it becomes necessary to identify the effective symmetry axis with the *average* chain orientation rather than an instantaneous chain orientation. The interpretation of the order parameter must then take into consideration chain reorientation in addition to the intramolecular motion. For the purpose of the present discussion we may assume that the intramolecular motion of the vector $\hat{\mathbf{r}}$ is also axially symmetric with respect to the instantaneous chain axis. We may then rewrite the order parameter as

$$\begin{aligned} S_\beta &= \frac{1}{2}\overline{(3\cos^2\beta - 1)} \\ &= \left[\frac{1}{2}\overline{(3\cos^2\alpha - 1)}\right]\left[\frac{1}{2}\overline{(3\cos^2\gamma - 1)}\right] \\ &= S_\alpha S_\gamma \end{aligned} \quad (3)$$

Here the angles are defined as illustrated in Figure 1. There are other requirements about the motions and their relative time scales which must be satisfied for the above result to be valid, but we defer further discussion of these conditions to a subsequent section. The important point to note at this time is that the order parameter measured by NMR can include contributions from both chain reorientation and chain isomerization, and these cannot be measured independently. Equation 3 expresses the order parameter as a simple product of S_α, a chain order parameter, and S_γ, an intramolecular order parameter. The NMR measurements always will yield this product, but, as we shall see, comparison of proton and deuterium order parameters can lead to an estimate of the relative importance of S_α and S_γ.

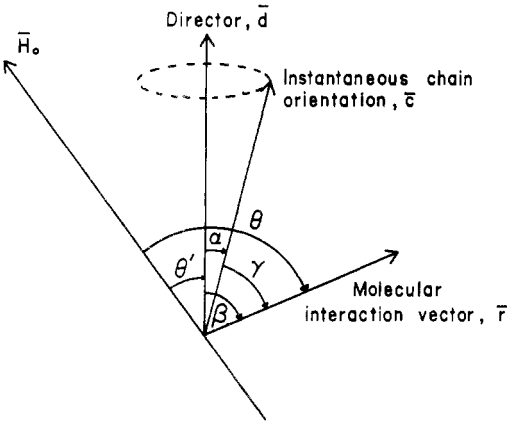

FIGURE 1: Illustration of the vectors and angles relevant to the lipid chain motional model. $\hat{\mathbf{H}}_0$ is the direction of the applied magnetic field. The director, $\hat{\mathbf{d}}$, is normal to the bilayer surface.

A Motional Model for Evaluation of the Order Parameter

The order parameter is a measure of the distribution of orientations of the molecular vector $\hat{\mathbf{r}}$ with respect to the director. Because of the axial symmetry the distribution function, $g(\beta)$, depends only on the angle, β, between the molecular vector and the director. If this distribution function can be deduced for a given model of the motions, then the order parameter can be calculated for this model by

$$S_\beta = \frac{\frac{1}{2}\int_0^\pi (3\cos^2\beta - 1)g(\beta)\sin\beta\, d\beta}{\int_0^\pi g(\beta)\sin\beta\, d\beta} \quad (4)$$

where the $\sin\beta$ factor takes into account the statistical weight corresponding to the number distribution of $\hat{\mathbf{r}}$'s about the director for a given β.

As a first approach to interpreting the NMR order parameter in lipid bilayer systems, Chan and co-workers (Seiter and Chan, 1973; Feigenson and Chan, 1974) have suggested a model in which the interproton vector of a methylene segment is considered to be freely moving through Brownian motion but only within an angular range of $\bar{\beta} \pm \Delta\beta$. Because of the axial symmetry, the average orientation corresponds to $\bar{\beta} = \pi/2$, and the distribution function for this model is

$$\begin{aligned} g(\beta) &= \text{constant for } \pi/2 - \Delta\beta \leq \beta \leq \pi/2 + \Delta\beta \\ &= 0 \text{ for } \beta \text{ outside the above range} \end{aligned} \quad (5)$$

Integration of eq 4 yields the value of the order parameter as a function of $\Delta\beta$ only, namely

$$S_\beta = -\frac{1}{2}\cos^2\Delta\beta \quad (6)$$

Although this model can allow for several types of motions, it does not lend insight into the detail of the motions which lead to the distribution function, and is therefore not wholly satisfying.

We now consider in detail the motional model in which the distribution of β is governed by two types of motions: (i) chain reorientation and (ii) chain isomerization. If the two motions are independent, that is, if the interconversion between trans and gauche methylene orientations does not depend on the instantaneous chain orientation, or vice versa, and if they occur on different time scales, we may describe the distribution function arising from each motion independently. From Figure

1 it is apparent that chain reorientation results in changes in the angle α characterized by a distribution function $g(\alpha)$, whereas chain isomerization results in changes in the angle γ, characterized by a distribution function $g(\gamma)$. The distribution function appearing in eq 4 can then be replaced by $g(\alpha)g(\gamma)$.

Following Seelig and co-workers (Seelig and Seelig, 1974a; Schindler and Seelig, 1975), we may assume the distribution function arising from chain isomerizations to be governed by the methylene segment orientation with respect to the instantaneous chain orientation. Therefore the distribution function is described by discrete values of γ, each with a population fraction $p(\gamma)$, i.e.,

$$g(\gamma) = p(\gamma_i)\delta(\gamma - \gamma_i) \tag{7}$$

with $\Sigma p(\gamma_i) = 1$. Chain reorientation may be a result of the entire molecule reorienting, possibly by cooperative motions within the bilayer. We consider it more likely, however, to be a result of individual chains tilting with respect to the bilayer possibly because of motions in the glycerol segments and the ester linkages. Because of the steric restrictions of neighboring chains, this tilting is likely to be locally cooperative, and to cover all angles within a certain range, say $\Delta\alpha$, and at a rate slow compared with that for chain isomerizations. It is therefore reasonable to approximate this motion to a random walk, and we propose

$$g(\alpha) = \text{constant for } 0 \leq \alpha \leq \Delta\alpha$$

$$g(\alpha) = 0 \text{ for } \alpha > \Delta\alpha \tag{8}$$

Note that for this distribution function, the *average* orientation of the chain is the director.

To evaluate the order parameter, we now express the function $(3\cos^2\beta - 1)$ in terms of α and γ. The relationship is given by the well-known addition theorem for spherical harmonics, namely

$$(3\cos^2\beta - 1) = \frac{1}{2}(3\cos^2\alpha - 1)(3\cos^2\gamma - 1) + \frac{3}{2}\sin^2\alpha\sin^2\gamma\cos 2\psi + \frac{3}{2}\sin 2\alpha\sin 2\gamma\cos\psi \tag{9}$$

where ψ is a phase angle of $\hat{\mathbf{r}}$ about the instantaneous chain orientation. Although chain isomerization cannot average the phase angle over all values, a combination of chain reorientation and rotation of the lipid molecule about its long axis should. The latter motion does not affect α or γ and therefore not the distribution functions proposed above. An effective averaging of the phase angle does, however, simplify eq 9 to

$$(3\cos^2\beta - 1) = \frac{1}{2}(3\cos^2\alpha - 1)(3\cos^2\gamma - 1) \tag{10}$$

With the above change in variables, the expression for the order parameter may be rewritten as

$$S_\beta = \frac{\frac{1}{4}\int_\alpha\int_\gamma (3\cos^2\alpha - 1)(3\cos^2\gamma - 1) \times g(\alpha)g(\gamma)\sin\alpha\sin\gamma\,d\gamma d\alpha}{\int_\alpha\int_\gamma g(\alpha)g(\gamma)\sin\alpha\sin\gamma\,d\gamma d\alpha} \tag{11}$$

In the present model, chain reorientation and chain isomerization are considered independent motions so that α and γ are independent variables. The integrations can therefore be performed independently, and with the choice of distribution functions proposed in eq 7 and 8, we obtain

$$S_\beta = \left[\frac{1}{2}\frac{\int_0^{\Delta\alpha}(3\cos^2\alpha - 1)\sin\alpha\,d\alpha}{\int_0^{\Delta\alpha}\sin\alpha\,d\alpha}\right] \times \left[\frac{1}{2}\sum_i p(\gamma_i)(3\cos^2\gamma_i - 1)\right] = S_\alpha S_\gamma \tag{12}$$

which is analogous to the expressions previously used by Niederberger and Seelig (1974) and Hubbell and McConnell (1971).

An important consequence of eq 12 is that the order parameter predicted from chain isomerization calculations would be scaled by a factor, S_α, which would be approximately constant for the whole chain. Thus the relative order parameter for various segments along the chain can probably be predicted quite well by models for chain isomerization, as has been attempted by several investigators (Marčelja, 1974; Schindler and Seelig, 1975; Jackson, 1976). The key difference between the present model and that proposed by Seelig and co-workers (Schindler and Seelig, 1975) and Marčelja (Marčelja, 1974) is the manner in which the scaling is performed. The latter workers are forced to invoke a rapid equilibrium among a distribution of orientations of the first methylene segment in order to fit their calculations to the observed order parameter values. As a result, the probabilities obtained for the various orientations of a segment relative to the bilayer normal depend strongly on the orientation of the first segment and the p_t values depend on the choice of the distribution. Although the equilibrium between orientations within the distribution must be fast on the NMR time scale as was pointed out by Schindler and Seelig (Schindler and Seelig, 1975), this interconversion must, we feel, be slow compared with the rate of chain isomerization. This is fundamentally a most important point, and, since we also argue that the chain reorientation is locally cooperative and involves a number of lipid molecules, p_t would be expected to be essentially independent of the chain orientation. This is the key difference between our present approach and previous ones.

We now calculate the order parameter expected in the various NMR experiments for the present model of molecular motion. The chain order parameter, S_α, depends only on the magnitude of the chain deflection, $\Delta\alpha$, and therefore contributes equally to the proton dipolar splitting and the deuterium quadrupolar splitting. The intramolecular order parameter, S_γ, depends on the population fraction of a given conformer, $p(\gamma)$, as well as the angle which the molecular vector makes with respect to the instantaneous chain direction. Since this angle is different for a C-D bond and the geminal H-H internuclear vector in some of the conformations, S_γ is in general different for the two measurements.

It is readily shown that

$$S_\alpha = \frac{1}{2}(\cos^2\Delta\alpha + \cos\Delta\alpha) \tag{13}$$

This function is illustrated in Figure 2. Unrestricted motion of the chain would require $\Delta\alpha = 180°$, and as expected S_α vanishes in this limit. In many liquid crystal systems the end-over-end orientation of the molecules is of little consequence either because of symmetry or because the two ends are similar

TABLE I: The Values of $(3\cos^2\gamma - 1)$ for Each of the Three Orientations of a Methylene Segment and for Each of the Magnetic Resonance Experiments.

Experiment	Interaction vector	Conformer: Trans γ	Trans $(3\cos^2\gamma - 1)$	Gauche + γ	Gauche + $(3\cos^2\gamma - 1)$	Gauche − γ	Gauche − $(3\cos^2\gamma - 1)$
^{1}H NMR	H→H	90°	−1	60°	−¼	120°	−¼
^{2}H NMR	C-D	90°	−1	90°	−1	35° 26′[a]	1
ESR	p orbital	0°	2	60°	−¼	60°	−¼

[a] Perfect tetrahedral symmetry has been assumed. Correction to the actual bond angles amounts to less than 1% in all cases.

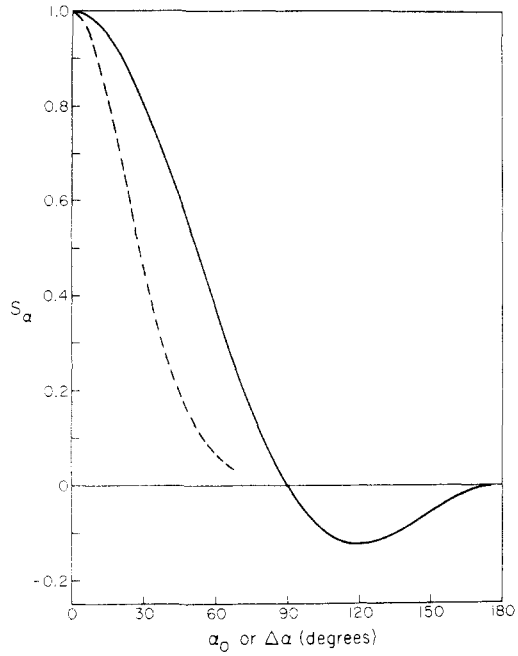

FIGURE 2: The chain reorientation order parameter, S_α, calculated as a function of the extent of chain fluctuation as measured by $\Delta\alpha$ (—) assuming a constant distribution for angles less than $\Delta\alpha$, or α_0 (- - -) assuming a normal distribution with a standard deviation of α_0.

in size and polarity. In those cases unrestricted motion is achieved when $\Delta\alpha \geq 90°$ since the orientation distribution function is symmetric about $\alpha = 90°$. This is not true of lipid molecules in the bilayers because of the amphiphilic nature of the molecules. In addition, unrestricted motion of the chains is not possible here, since they are anchored at the glycerol segments. It is, however, still possible to obtain small values for S_α as $\Delta\alpha$ tends toward 90° (cf. Figure 2).

Chain isomerization is characterized by an averaging of the methylene segment orientation arising from a rapid interconversion among distinct conformers. There are three likely conformations: trans, gauche-plus, and gauche-minus. Because of symmetry, the two gauche populations must be equal and as a consequence the normalization condition is

$$\sum p(\gamma_i) = p_t + 2p_g = 1 \qquad (14)$$

The angle, γ_i, between the molecular interaction vector $\vec{r}$ and the instantaneous chain orientation, depends, for a given conformer, on which magnetic resonance experiment is being employed to probe the motion. The proton-proton dipolar interaction depends on the orientation of the geminal interproton vector, whereas the deuterium quadrupolar interaction is sensitive to the C-D bond orientation. The usual acyl chain nitroxide spin label (Hubbell and McConnell, 1971) contains the unpaired electron in a p-orbital which is normal to the methylene segment plane, and the ESR spectrum depends on the average orientation of the p-orbital. The values of the angles, γ_i, and the function $(3\cos^2\gamma_i - 1)$, summarized in Table I, for each of the conformers and for each of the magnetic resonance experiments under consideration yield the following intramolecular order parameters:

$$\text{(i) } S_\gamma = -\frac{3}{8}p_t - \frac{1}{8} \quad \text{for the nuclear dipolar interaction}$$

$$\text{(ii) } S_\gamma = -\frac{1}{2}p_t \quad \text{for the deuterium quadrupolar interaction} \qquad (15)$$

$$\text{(iii) } S_\gamma = \frac{9}{8}p_t - \frac{1}{8} \quad \text{for the acyl-chain nitroxide radical}$$

Seelig and Seelig (1974a) have pointed out that a segment in a trans conformation may appear as if it were in a gauche conformation whenever there is a *single* gauche rotation at a segment further up along the chain. For this reason, p_t would provide a lower limit of the true probability of a segment being in a trans conformation. In the present analysis, however, *single* trans-gauche rotations in the upper part of the chain can be considered to contribute to the chain reorientation order parameter since the motion is likely to be on a time scale comparable to the chain reorientation time scale as the same steric restrictions will probably prevail. The present p_t value should therefore not be very different from the real value. Moreover, the larger the value of p_t, the smaller the contribution from single trans-gauche rotations to S_α or S_γ would be.

The experimental order parameters, denoted S_{HH}, S_{CD}, and S_{ESR}, may be compared directly via eq 15, provided S_α can be assumed to have the same value for each of these experiments. For instance, one can readily show that $S_{ESR} = -\frac{9}{4}S_{CD} - \frac{1}{8}$. It has been customary to define a molecular order parameter, S_{mol}, for methylene segments, such that the angle β is taken between the director and the normal to the methylene plane, and for the ESR spin label discussed here $S_{mol} = S_{ESR}$. In general, however, the experimental order parameters are related to S_{mol} only through equations analogous to eq 15. Note that the common transformation $S_{mol} = -2S_{CD}$ is only valid in the limit of $p_t \to 1$.

However, it is possible, that S_α differs between S_{CD} and S_{ESR}. This possibility arises because the time scale of observation for an ESR experiment is much shorter than that for an NMR experiment, and as a consequence, the two techniques are likely to sample the chain reorientation motion differently.

It is probable that the ESR experiments will then measure an instantaneous but restricted powder distribution of chain orientations, even in oriented samples. In fact, as we will discuss later, this might be the origin of the spectral effects which McConnell and co-workers have attributed to bent chains (McFarland and McConnell, 1971).

In contrast the time scales of observation for the two NMR measurements are comparable, and the contribution to the respective order parameters from S_α should be the same. A comparison of S_{HH} and S_{CD} can then be used to get an estimate of p_t. The experimental values for S_{CD} for the first ten carbon segments are always quite similar, and, for the bilayer in the liquid crystalline phase, the average value is about -0.20 ± 0.01 (Seelig and Seelig, 1974a; Stockton et al., 1976). Thus about two-thirds of the methylene segments have S_{CD} values of this magnitude, the remaining being closer to zero. The ^1H NMR order parameter is deduced by simulating the early part of the free induction decay[2] and is, therefore, an average value for those segments with the largest absolute value of the order parameter. This implies that the average S_{CD} and the S_{HH} deduced this way, measure the same portion of the chain, and it is valid to compare them. The S_{HH} value inferred from the $\Delta\beta$ values required to fit the early part of the free induction decay (Seiter and Chan, 1973) is -0.17 ± 0.04. The order parameter S_{CD} is nearly equal to or larger than S_{HH}, which by eq 15 indicates that p_t must be close to unity, and we conclude that a major contribution to the reduction of the order parameter in these systems arises from chain reorientation. This is illustrated more clearly in Figure 3, which shows the range of p_t values that will satisfy either the condition that $S_{CD} = -0.20 \pm 0.01$ or the condition that $S_{HH} = -0.17 \pm 0.04$ for a given $\Delta\alpha$. The region of overlap (indicated by the hatched area in Figure 3) corresponds to the combinations of p_t and $\Delta\alpha$ values which will satisfy both conditions simultaneously. For the bilayer membrane in the liquid-crystalline phase, the ranges of p_t and $\Delta\alpha$ are therefore

$$1 \geq p_t \geq 0.8 \text{ with } -0.5 \leq S_\gamma^{CD} \leq -0.40$$
$$\text{or } -0.5 \leq S_\gamma^{HH} \leq -0.43 \qquad (16)$$
$$\text{and } 60° \geq \Delta\alpha \geq 50° \text{ with } 0.38 \leq S_\alpha \leq 0.53$$

It is interesting to note that the deuterium order parameters measured for the lyotropic liquid-crystalline phase of long chain fatty acids are about 50% larger than those obtained for the lipid bilayer (Niederberger and Seelig, 1974), suggesting a more ordered phase in the former. Seelig and Niederberger (1974b) estimated a value for S_α of about 0.7–0.8, corresponding to a $\Delta\alpha$ of about 35°. The principal difference between the two systems is therefore a substantially smaller angular excursion of the chain in the case of the fatty acids presumably because of a more regular packing of the acyl chains in their liquid-crystalline phase.

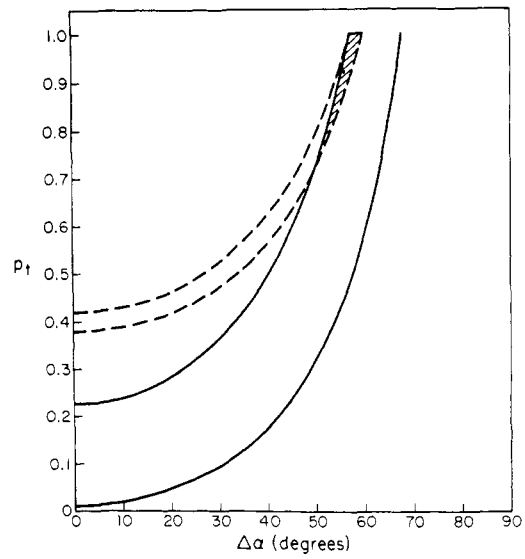

FIGURE 3: The range of values of p_t, the probability of a trans orientation, for given values of $\Delta\alpha$, the limit of the chain fluctuation, which yields (i) a deuterium order parameter, $S_{CD} = -0.2 \pm 0.01$ (- - -) or (ii) a proton order parameter $S_{HH} = -0.17 \pm 0.04$ (—). The region of overlap (////) corresponds to the combination of p_t and $\Delta\alpha$ values which yield both $S_{CD} = -0.2 \pm 0.01$ and $S_{HH} = -0.17 \pm 0.04$.

The choice of a constant distribution of angles α within the range of $\Delta\alpha$ (cf. eq 8) physically corresponds to a complete freedom of movement of the chains within the specified restriction barriers. This is clearly an approximation, and angular distributions which more closely reflect the actual physical state of the bilayer could be employed, if the manner in which the chain reorientation comes about was better understood. Note, however, that the choice of the distribution of α does not alter the basic conclusion, derived from Figure 3, that S_α must be in the range of 0.38–0.53, but merely reflects our view of *how* the chain reorientation motion leads to this value of S_α. As another illustrative example we can consider the case where there is a normal distribution of chain reorientations about the director with a standard deviation given by the angle α_0. If we assume that the angular range of α can be extended to include the whole domain, i.e., $0 \leq \alpha \leq \pi$, the only parameter affecting the order parameter, S_α, is the standard deviation, α_0, and we can ascertain that value of α_0 which fits the observed S_α. With the distribution function written as

$$g(\alpha) = \text{constant } e^{-(\alpha/\alpha_0)^2/2} \qquad (17)$$

the order parameter, S_α, is given by

$$S_\alpha = \frac{\frac{1}{2}\int_0^\pi (3\cos^2\alpha - 1)e^{-(\alpha/\alpha_0)^2/2} \sin\alpha \, d\alpha}{\int_0^\pi e^{-(\alpha/\alpha_0)^2/2} \sin\alpha \, d\alpha} \qquad (18)$$

This order parameter is plotted vs. α_0 in Figure 2. Since S_α has to fall in the range 0.38–0.53 to satisfy the ^1H and ^2H NMR order parameter data, it follows that the corresponding range of α_0 would be 27–34°. These values of α_0 are naturally much smaller than the $\Delta\alpha$ values obtained with the constant distribution used above, because $\Delta\alpha$ is the *limit* of the range of values that α can assume whereas α_0 in the normal distribution corresponds approximately to the most probable value of α. To illustrate this point, we have plotted in Figure 4 both distribution functions for the appropriate value of $\Delta\alpha$ or α_0 which yields the observed experimental order parameters. A more meaningful comparison to make, perhaps, is that between the

[2] The geminal intraproton pair order parameter, S_{HH}, was obtained by computer simulation of the early part of the free induction decay assuming that the geminal dipolar interaction dominates. The simulation was also performed allowing fully for interpair (both intrachain and interchain) dipolar interactions, but these latter contributions were found to be small, affecting S_{HH} no more than about 10% for a given $\Delta\beta$. In the computer simulations the interproton vector within the geminal pair was allowed to fluctuate freely within a specified range of angles, $\pm\Delta\beta$. The angle of fluctuation was then obtained by direct comparison of calculated and experimental free induction decays. The best fit was obtained for $\Delta\beta \sim 55 \pm 5°$ from which the order parameter was estimated to be in the range $S_{HH} = -0.17 \pm 0.04$. The error reflects partly the uncertainty in the interpair and interchain contributions.

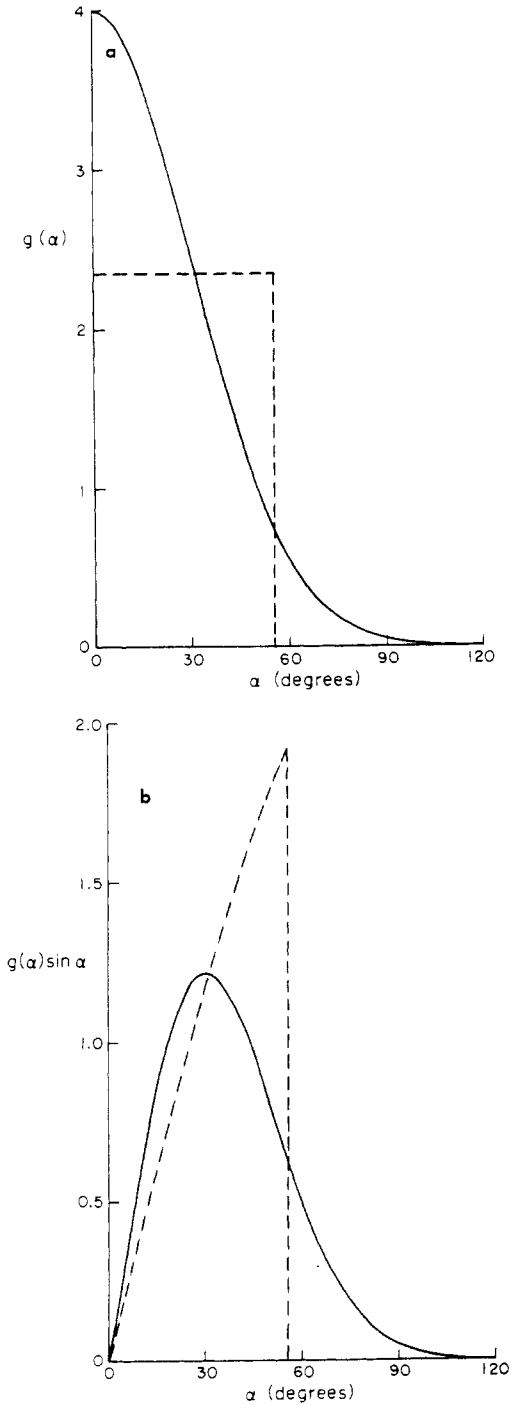

FIGURE 4: (a) The distribution functions $g(\alpha)$ discussed in the text for a constant distribution within the angular range $0 \leq \alpha \leq 55°$ (- - -) (i.e., $\Delta\alpha = 55°$) and for a normal distribution with a standard deviation $\alpha_0 = 30°$ (—). (b) The normalized weighted distributions $g(\alpha)$ sin α for a constant distribution function $g(\alpha)$ with $\Delta\alpha = 55°$ (- - -) and a normal distribution with $\alpha_0 = 30°$ (—). These distribution functions yield an order parameter value $S_\alpha = 0.45$.

weighted distribution functions $g(\alpha)$ sin α (cf. Figure 4), since it is the weighted distribution function which determines the composite ESR powder spectrum observed in the oriented spin label studies. In fact, it is those chains with instantaneous orientation close to the most probable orientation or the mean orientation which determine the gross features of the ESR spectrum. The shape of these weighted distribution functions clearly demonstrates that the most probable angle is nonzero. One important consequence of this result is that the ESR spectra can be interpreted in terms of permanently tilted chains (Gaffney and McConnell, 1974; Birrell and Griffith, 1976). However, since the same spectral effects can arise from a statistical distribution of chains when the distribution is sampled on a short time scale, it is more correct, in our view, to interpret the tilt in a dynamic sense. Although our present conclusions based on NMR data are in substantial agreement with the ESR spin label results, the NMR method admittedly cannot distinguish between a normal distribution of chains about the director vs. a normal distribution about a permanently tilted orientation provided that the chains rotate about the director in the latter case.

Our present conclusion that chain isomerization is not the only important motion in lipid bilayers is in agreement with the data from previously reported x-ray diffraction studies (Wilkins et al., 1971) and recent Raman spectroscopic studies (Mendelsohn et al., 1976; Gaber and Peticolas, 1976). The electron density profile of the lipid bilayer in the gel phase shows a deep electron-deficient trough at the center of the bilayer. This arises from the terminal methyl groups of the chain. When the bilayer is heated to the liquid-crystalline phase, the previously sharp trough broadens and becomes more shallow, which has been interpreted to mean that the methyl groups are localized over a larger range of the bilayer. This could be accounted for by a chain reorientation motion, and although chain isomerizations would give rise to a chain shortening, and therefore a possible delocalization of the methyl group, the large p_t value suggests a low probability of many simultaneous trans-gauche rotations in a given chain, and hence any delocalization effect due to chain isomerization should be fairly minimal.

The recent detailed interpretations of Raman spectra obtained from lipid bilayers enabled these investigators to assess quantitatively the changes with temperature of both the intramolecular and the intermolecular order. These workers observed large changes in both quantities upon passing from the gel to the liquid-crystalline phase. The intramolecular order determined for the liquid crystalline phase is compatible with a large p_t value, and the observed change in the intermolecular order is consistent with increased chain reorientation above the phase transition temperature.

Dynamic Behavior

Although the order parameter provides a measure of the degree of motional restriction, the motional state of the bilayer system is only fully defined when both the motional distribution function and the time scales of the molecular motions are ascertained. In the previous section we propose a motional model to account for the magnetic resonance order parameters. The purpose of the present section is to illustrate how the same motional model leads naturally to an interpretation of NMR relaxation measurements, which have previously been undertaken to elucidate the dynamic behavior of the system (Feigenson and Chan, 1974; Horwitz et al., 1972; McLaughlin et al., 1973; Lee et al., 1973).

Proton and carbon-13 spin-lattice relaxation times of lipid bilayer membranes have been shown to increase with both increasing temperature and increasing frequency of irradiation. These findings can only be accounted for if the dipolar interactions responsible for the spin-lattice relaxation are modulated by at least two motions with correlation times, $\tau_\parallel$ and $\tau_\perp$, such that $(\omega_0\tau_\parallel)^2 \ll 1$ and $(\omega_0\tau_\perp)^2 \gg 1$. Under these conditions, the spin-lattice relaxation rate may be approximated by (Kroon et al., 1976)

$$\left(\frac{1}{T_1}\right) \cong A\tau_{\parallel} + B\frac{1}{\omega_0^2\tau_{\perp}} \tag{19}$$

with the first term dominating the observed temperature dependence, and the second term responsible for the observed frequency dependence. On the basis of proton T_1 studies, Feigenson and Chan (1974) have estimated the following ranges of correlation times for the liquid-crystalline phase:

$$\tau_{\parallel} \approx 10^{-9}\text{–}2 \times 10^{-10}\ \text{s}$$

$$\tau_{\perp} \geq 10^{-7}\ \text{s} \tag{20}$$

However, the origins of these time scales were not fully understood at that time. We suggest now that the faster time scale, $\tau_{\parallel}$, is associated with rotational isomerization whereas the slower time scale, $\tau_{\perp}$, reflects primarily the rate of chain reorientation. For the bilayer systems under consideration here, these time scales are sufficiently different that the expansion of the order parameter as presented above would be valid.

It has been suggested (Träuble, 1971) that most trans-gauche rotations in the lipid chains occur as β-coupled trans-gauche $\mp$ rotations giving rise to a kink, rather than as isolated events. This suggestion is based on the argument that a kink is energetically favored over isolated trans-gauche rotations, because there are less unfavorable interactions with neighboring chains. The formation and subsequent disappearance of a kink is therefore one mechanism whereby the dipolar interaction can be modulated (Kroon et al., 1976; Kimmich and Peters, 1975). Examination of models further suggests that a kink also may be displaced along the chain by a set of δ-coupled rotations around the kink as illustrated in Figure 5. A kink may conveniently be denoted by $k^{\pm}_n$, where the subscript denotes the carbon atom closest to the acyl end of the chain, such that the first trans-gauche rotation in the kink occurs around the bond between carbon n and $n+1$, and the superscript denotes the sense of that bond rotation (Flory, 1969). The δ-coupled rotations then transform an even kink to another even kink but with opposite sense. That is, a kink diffusion corresponds to an interconversion among even or odd kinks with alternating sense, e.g.,

$$\ldots k^{+}_{n-4} \rightleftharpoons k^{-}_{n-2} \rightleftharpoons k^{+}_{n} \rightleftharpoons k^{-}_{n+2} \rightleftharpoons k^{+}_{n+4} \ldots$$

There are therefore in effect four subsets of kinks, with interconversion among the subsets only possible either (i) through a totally eclipsed conformation, which is energetically very unfavorable, or (ii) by kink annihilation to form the all-trans chain followed by formation of a kink of a different sense or at a new position. It is reasonable to argue that the formation of a kink is most likely to occur toward the terminal end of the chain where the least steric hindrance is present. Once formed, the kink would diffuse along the chain, passing through each methylene segment many times as it undergoes a simple random walk type motion. The lifetime of a kink is determined by the number of times the kink is reflected at the two ends of the chains before it ultimately is dissipated at the terminal end of the chain. In this diffusion process, the correlation time $\tau_{\parallel}$ is that time scale associated with the time between a kink leaving and then returning to a given segment, and in the meantime having undergone the random walk along the chain. Clearly $\tau_{\parallel} \leq \tau_{\text{lifetime}}$. One might expect $\tau_{\parallel}$ to vary somewhat with the location of the methylene segment in the chain. In particular, $\tau_{\parallel}$ should be shorter the closer the segment is to that end of the chain which serves as the kink sink. Proton and carbon-13 spin-lattice relaxation measurements on *sonicated* bilayer membranes have revealed a gradient in the relaxation times

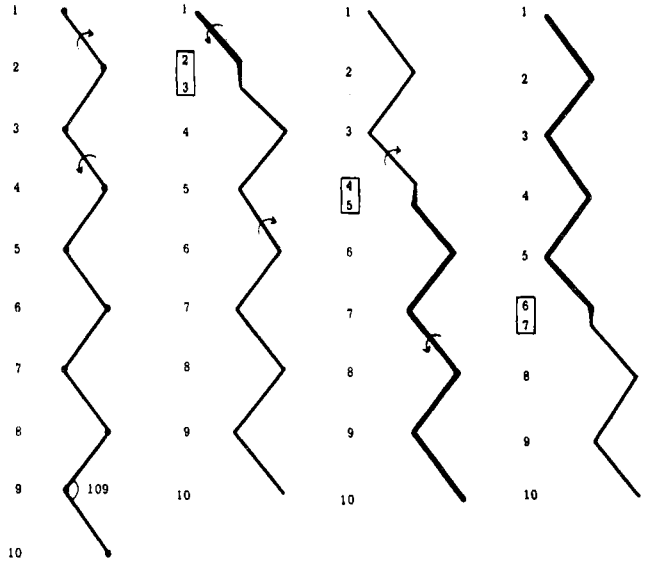

FIGURE 5: Schematic illustration of δ-coupled trans-gauche $\pm$ rotations around a kink leading to kink diffusion. The arrows indicate the sense of the rotations keeping the higher numbered carbon fixed, and the particular sequence illustrated corresponds to, from left to right: all-trans $\rightarrow k_1^{+} \rightarrow k_3^{-} \rightarrow k_5^{+}$. The carbon atoms are numbered, and the pair of methylene segments which are oriented off-axis with respect to the chain are indicated by the rectangle around the carbon numbers. It is evident that this kink diffusion sequence will affect every methylene segment along the chain.

along the chain in the direction expected (McLaughlin et al., 1973; Lee et al., 1973) and these observations could well have origin in this variation of $\tau_{\parallel}$ along the hydrocarbon chain.[3]

The value of $\tau_{\parallel}$ for the upper part of the chain may be estimated on the basis of simple one-dimensional diffusion theory as follows. Once a kink is formed, the jump rate, i.e., the rate at which the kink becomes displaced by one unit length l, is given by

$$1/\tau_{\text{jump}} = Ae^{-\Delta E/RT} \tag{21}$$

Here A is the frequency factor ($\sim 10^{14}\ \text{s}^{-1}$) and ΔE, the activation energy for the δ-coupled rotation, which we estimated to be ~3–4 kcal/mol of kinks (Kroon et al., 1976). The jump time is then approximately 10^{-12} to 10^{-11} s. Since the mean square distance $\langle l^2 \rangle$ travelled in the time $\tau_{\parallel}$ is

$$\langle l^2 \rangle = 2\frac{l^2}{\tau_{\text{jump}}}\tau_{\parallel} \tag{22}$$

$\tau_{\parallel}$ may be estimated to be of the order of 10^{-10} s, which is the order of magnitude inferred from the proton T_1 data.

The kink diffusion model would also suggest that the frequency with which a kink appears in a certain position would be sensitive to the total number of available positions for the kink. The model therefore predicts that $\tau_{\parallel}$ and therefore T_1 should depend on the chain length. In particular, a longer chain would have more possible kink positions, resulting in a longer kink diffusion time and therefore an enhanced spin-lattice relaxation rate (a shorter T_1). Indeed Kainosho et al. (1977) have shown that for sonicated vesicles ($1/T_1$) increases as the chain length increases from a total of 12 to 18 carbon atoms.

[3] Proton and carbon-13 spin-lattice relaxation measurements of chain resonances in sonicated bilayer membranes show a temperature and frequency dependence analogous to that found in unsonicated systems (Kroon et al., 1976; Godici and Landsberger, 1975). The chain motions which dominate T_1 are therefore similar for the two bilayer systems.

If the slower correlation time, $\tau_\perp$, for multilayers arises from fluctuations of the tilt of the chain with respect to the bilayer normal, then $\tau_\perp^{-1}$ measures the rate at which the chain traverses the angular range $\Delta\alpha$. In general, one expects $\Delta\alpha$ and $\tau_\perp$ to exhibit a complex dependence on the property of the bilayer, particularly since the chain reorientation could be partly molecular and partly cooperative in its origin. If the chain reorientation is principally molecular and controlled by rotational diffusion processes, then one expects the rate of chain reorientation to be inversely proportional to the amplitude of the angular fluctuation; that is, the larger $\Delta\alpha$, the larger $\tau_\perp$ is expected to be. On the contrary, if chain reorientation is principally cooperative, involving say several hundred lipid molecules, then one expects a distribution of correlation times with both a high- and a low-frequency cut-off. The former is expected to be controlled by the single molecule reorientation rate, and the latter should depend on the nature of the cooperative unit. The measured $\tau_\perp$ really then reflects the most effective combination of timescales and amplitudes of the chain reorientation for the spin–lattice relaxation process. Thus if the size of the cooperative unit decreases as is likely to happen as the bilayer becomes less ordered, the effective correlation time would be expected to decrease.

Effect of Sonication

The effect of sonication is to produce bilayer units, namely, vesicles, which are sufficiently small that the overall tumbling of the bilayer unit can contribute to averaging of the residual first-order dipolar or quadrupolar interactions (Seiter and Chan, 1973). If the vesicles are small enough (<500 Å in diameter), the vesicle tumbling rate should be rapid enough that the observed proton or deuterium NMR line widths become homogeneous; i.e., they are the same for all molecules within the bilayer vesicle. Under these circumstances the proton line width for a methylene segment which is undergoing the restricted anisotropic motion described in the model presented in the previous section may be expressed by (Hagan et al., 1977)

$$\Delta\nu_H = \frac{1}{\pi T_2} = \frac{4}{5\pi}\left(\frac{3\gamma^2\hbar}{4r^3}\right)^2 (S_\gamma^{HH})^2 \left\{S_\alpha^2\tau_e + \frac{1}{9}\left(\frac{5}{2}\sigma_\alpha^2 - 5S_\alpha^2 - 2S_\alpha + 9\mu_\alpha^2 + 7\right)\tau_\perp\right\} \quad (23)$$

where S_γ^{HH} and S_α are the order parameters defined in the previous section, μ_α is the mean of the function $\sin 2\alpha(t)$, and σ_α^2 is the variance associated with the molecular axis reorientation relative to the director so $\sigma_\alpha^2 = \overline{(3/2\cos^2\alpha(t) - 1/2)^2} - S_\alpha^2$. For proton dipolar interactions within a methylene segment $(3\gamma^2\hbar/4r^3)^2 = 9.7\times10^9\ s^{-2}$. The correlation times in eq 23 are the effective correlation times associated with modulation of the dipolar interactions and $(1/\tau_e) = (1/\tau_v) + (1/\tau_{ld})$ where $1/\tau_v$ is the rate of vesicle tumbling and $1/\tau_{ld}$ is the rate of lateral diffusion of a lipid molecule on the spherical vesicle surface. It is assumed in eq 23 that the correlation time, $\tau_\perp$, associated with the chain reorientation relative to the director is comparable to that of the chain reorientation about the director, and that this time is at least an order of magnitude faster than τ_e.

It is apparent from the form of eq 23 that there are two contributions to the homogeneous line width, (i) the spin–spin relaxation due to chain motion, and (ii) the modulation of the residual dipolar interactions by vesicle tumbling together with lateral diffusion. A third contribution to the line width not included here arises from the spin–lattice relaxation, but, since $(1/T_2) \gg (1/T_1)$ for anisotropic motion, it could be ignored. Note that, if chain reorientation relative to the director is absent, the second term in the line width expression of eq 23 is identically zero and

$$\Delta\nu = \frac{4}{5\pi}\left(\frac{3\gamma^2\hbar}{4r^3}\right)^2 S_{HH}^2\tau_e \quad (24)$$

This expression is commonly employed for the calculation of the line width for sonicated vesicles (Bloom et al., 1975). We contend, however, that this approximation is not justified for the bilayer vesicle system, because of the substantial chain reorientation motion relative to the director even in the multilayer system.

The derivation by Hagan et al. (1977) resulting in eq 23 is strictly valid only for isolated pairs of protons, and eq 23 does not account for the interpair dipolar interactions in the fatty acid chain. However, the theory is exact for the deuterium line width observed in ^2H NMR experiments where the line width is controlled by the modulation of the quadrupolar interaction. To calculate the deuterium line width, it is only necessary to introduce into the treatment the appropriate interaction constant and intramolecular order parameter. Thus

$$\Delta\nu_D = \frac{9\pi}{20}\left(\frac{e^2qQ}{h}\right)^2 (S_\gamma^{CD})^2 \left\{S_\alpha^2\tau_e + \frac{1}{9}\left(\frac{5}{2}\sigma_\alpha^2 - 5S_\alpha^2 - 2S_\alpha + 9\mu_\alpha^2 + 7\right)\tau_\perp\right\} \quad (25)$$

We note that this line width expression reduces to that previously proposed by Stockton et al. (1976)

$$\Delta\nu_D = \frac{9\pi}{20}\left(\frac{e^2qQ}{h}\right)^2 S_{CD}^2\tau_v + \frac{1}{\pi T_1} \quad (26)$$

only in the limit where chain reorientation and lateral diffusion are not important motions. Equations 23 and 25 predict that the ratio of the calculated proton and deuterium line widths is just the ratio of the interaction constants multiplied by the ratio of the intramolecular order parameters. On this basis one expects $(\Delta\nu_H/\Delta\nu_D) = 0.016(S_\gamma^{HH}/S_\gamma^{CD})^2$, which is about 0.016 since S_γ^{HH} and S_γ^{CD} are about equal. The observed proton line widths are on the order of 20–30 Hz when the full methylene intensity is observed for the composite methylene signal (Lichtenberg et al., 1975). This is true also for the signal due to the α-CH_2's adjacent to the carbonyls of the fatty acid chains! The corresponding deuterium line widths (for the upper part of the chain at comparable temperatures) are about 550 ± 50 Hz (Stockton et al., 1976). The observed line width ratio is therefore about 0.036 which indicates that the proton line width of 20 Hz is indeed an upper limit of the true value. This is reasonable since the dispersion of chemical shifts for the protons along the chain as well as spin–spin coupling will tend to make the resonances appear broader.

Stockton et al. (1976) have measured both the deuterium line widths of vesicles and the order parameters in multilayers for the same lipid system and as a function of position of the deuterium along the chain. They found that the vesicle line widths for different positions vary linearly with the square of the multilayer order parameter measured for the same position in the chain and argued on the basis of this correlation that there is no reduction in the order parameter upon sonication. Equation 25 predicts this correlation if (i) there is no change in the intramolecular order parameter, S_γ^{CD}, upon sonication, or (ii) if the flexibility gradient changes proportionally along the chain. The important conclusion is that this correlation is independent of the value of S_α, or changes in it, since S_α applies to the whole chain.

If there is no structural perturbation of the lipid bilayer membrane upon sonication, then, on the basis of the order parameters which have been measured for lecithin multilayers, we expect the homogeneous line widths observed for small sonicated vesicles to be determined by the rate of vesicle tumbling, or the rate of lateral diffusion of the lipid molecules, or some combination of both motions. Although there have been numerous attempts to ascertain the dependence of the vesicle proton line widths upon solution viscosity (Horwitz et al., 1972; Sheetz and Chan, 1972; Lichtenberg et al., 1975; Bloom, personal communication), none, in fact, has been observed. This could mean that the effect of vesicle tumbling on the line width is small compared with the effect of lateral diffusion, i.e., $1/\tau_e \simeq 1/\tau_{ld}$. This possibility is not supported by the current estimates for the lateral diffusion coefficient of lipids in multilayers of 10^{-8} cm^2/s (Edidin, 1974). It is possible that this diffusion is in fact much faster in the vesicular bilayer. But McLaughlin (1976) has recently also obtained estimates of 10^{-8} cm^2/s for this lateral diffusion coefficient in lecithin bilayer vesicles. For these reasons, we have to conclude that the proton and deuterium line widths of small vesicles are controlled by the modulation of the dipolar interaction by local motions; that is, the second term of eq 23 and 25 dominates the total line width. Also, their ^{31}P NMR studies of bilayer vesicles showed that the ^{31}P line widths of the glycerol phosphate moiety in these systems exhibit a viscosity dependence, indicating that the chemical shift anisotropy mechanism which contributes to these line widths is modulated at least in part by overall tumbling of the bilayer unit. This important observation is to be contrasted with the lack of any detectable viscosity dependence in the proton spectrum.

If we use the model outlined in the previous section for which the distribution of chain reorientations relative to the director is uniform within an angular range from zero to $\Delta\alpha$, we can rewrite eq 23 or 25 as

$$\Delta\nu = C\{A(\Delta\alpha)\tau_e + B(\Delta\alpha)\tau_\perp\} \qquad (27)$$

where

$$A(\Delta\alpha) = S_\alpha{}^2 = \frac{1}{4}(\cos^2\Delta\alpha + 2\cos^3\Delta\alpha + \cos^4\Delta\alpha)$$

and

$$B(\Delta\alpha) = \frac{1}{72}(92 + 55\cos\Delta\alpha - 24\cos^2\Delta\alpha - 85\cos^3\Delta\alpha - 38\cos^4\Delta\alpha)$$

The constant C is either

$$\frac{4}{5\pi}\left(\frac{3\gamma^2\hbar}{4r^3}\right)^2 (S_\gamma{}^{HH})^2$$

or

$$\frac{9\pi}{20}\left(\frac{e^2qQ}{h}\right)^2 (S_\gamma{}^{CD})^2$$

depending on whether eq 27 refers to the proton or deuterium line width. The apparent lack of viscosity dependence implies that the second term dominates and, since τ_e is apparently dominated by τ_v, we expect

$$B(\Delta\alpha)\tau_\perp \geq A(\Delta\alpha)\tau_v \qquad (28)$$

The equality represents an upper limit for $A(\Delta\alpha)\tau_v$ since we would expect that, if 50% of the line width is viscosity dependent, it would be detectable. Figure 6 shows the dependence of $\Delta\nu/C\tau_v = A(\Delta\alpha) + B(\Delta\alpha)(\tau_\perp/\tau_v)$ on $\Delta\alpha$ for various ratios of $\tau_\perp/\tau_v$. The solid lines refer to that part of the function where eq 28 is applicable, that is, where there is no or only a small viscosity dependence. For a line width of 20–30 Hz and reasonable values for τ_v (5×10^{-6} to 5×10^{-7}), we see no viscosity dependence only if $\Delta\alpha > 70$ and if $\tau_\perp/\tau_v$ is small. The vesicle line width and its lack of viscosity dependence are therefore indicative of a significant reduction (by a factor of 2–3) in the order parameter. We point out that these arguments give an underestimate of $\Delta\alpha$ in part because the proton line width of eq 23 is an underestimate of the true line width. The dipolar interaction due to the other protons on the chain would lead to further broadening of the signal. It would be of interest to carry out a similar analysis with the deuterium line widths. Unfortunately the viscosity dependence here is not known. When such data become available, the relative importance of vesicle tumbling and local motion in the motional averaging can then be ascertained more precisely.

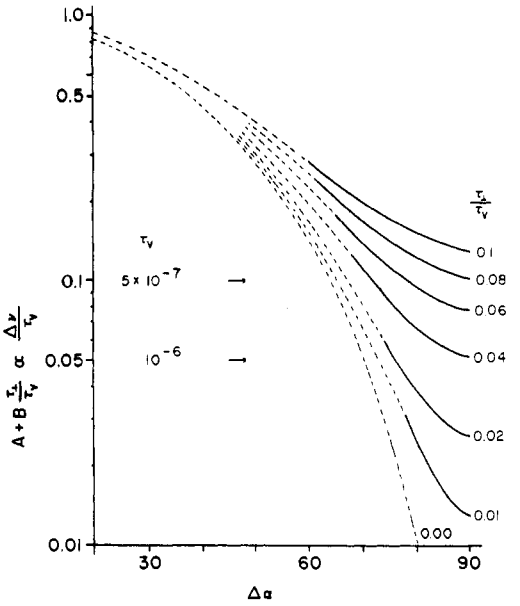

FIGURE 6: The function $A(\Delta\alpha) + B(\Delta\alpha)(\tau_\perp/\tau_v) = \Delta\nu/C\tau_v$ (cf. eq 27) as a function of $\Delta\alpha$ and for a series of values of $\tau_\perp/\tau_v$. The solid portion of the curves corresponds to combinations of $\Delta\alpha$ and $\tau_\perp/\tau_v$ for which $B(\Delta\alpha)\tau_\perp \geq A(\Delta\alpha)\tau_v$, the region over which little or no viscosity dependence of the line width should be observed. The correlation time for vesicle tumbling, τ_v, is expected to be within the range indicated based on the following experimental findings. For a 220-Å diameter vesicle in aqueous suspension at 20 °C, the Stokes–Einstein relationship predicts $D_{20,w} = 1.9 \times 10^{-7}$ cm^2/s. The measured value for fractionated egg yolk lecithin is $D_{20,w} = 1.9 \times 10^{-7}$ cm^2/s (Huang, 1969). At 50 °C, this value corresponds to a $D_{50,w} = 3.9 \times 10^{-7}$ cm^2/s which leads to a tumbling correlation time of $\tau_{50°C} = \frac{2}{9}R^2/D \sim 10^{-6}$ s. The lateral diffusion correlation time is approximately $\tau_{ld} = R^2/4D_{ld} = 3 \times 10^{-5}$ s.

In summary, the available NMR data on sonicated lipid systems at 50 °C suggest that upon sonication (i) the order parameter is reduced by a factor of 2–3 to a value about −0.07 to −0.1. (ii) The order parameter is reduced primarily through S_α, thereby causing all the order parameters along the chain to decrease by a near constant factor. (iii) The correlation time $\tau_\perp$ for chain reorientation is decreased from $\sim 10^{-7}$ to 10^{-8}–10^{-9} s.

Recent Raman spectroscopic studies (Mendelsohn, 1976; Gaber and Peticolas, 1976) have shown that well above the transition temperature there are no differences between the unsonicated and sonicated bilayer systems in their intramolecular order, as the intensities of those Raman lines which are proportional to the average number of extended all-trans chain

segments were found to be essentially identical for the two systems. This is compatible with the S_γ value being comparable for the two model membranes. In contrast, the Raman spectra show pronounced intensity differences in that region of the spectra which has been interpreted to measure intermolecular or interchain interactions. This reduction of intermolecular coupling of Raman active CH vibrations is compatible with an increase in the amplitude ($\Delta\alpha$) of chain reorientation as a result of the increase in surface curvature. The Raman data therefore support our present interpretation of the magnetic resonance data in terms of the larger amplitudes of chain reorientation with increasing surface curvature.

Conclusions

We have demonstrated that it is necessary to interpret the observed NMR order parameters in lipid bilayers in terms of chain reorientation motions in addition to isomerization of the chain. For the bilayer membrane in the liquid crystalline phase, the order parameter can be written as a product of two independent order parameters representing the two individual motions. We emphasize that the NMR experiments will always measure the product. This is to be contrasted with the ESR spin label experiments, where the spectra are primarily sensitive to chain isomerization because of the short time scale of measurement, or Raman spectroscopic studies, where inter- and intramolecular order can be inferred separately. Clearly a comparison of the state of chain motion deduced from the various techniques should be made only when the inherent differences in the measurements are fully appreciated.

A detailed analysis of the observed proton and deuterium order parameters indicates that the chain has considerable intramolecular order ($p_t > 0.8$) apart from the region at the tail of the chain. All told there is on the average less than one kink per chain in the upper portion of the chain. The high p_t value arrived at here is consistent with that derived by Hubbell and McConnell (1971) on the basis of their spin-label measurements, but the variation in p_t along the upper part of the chain must be small in accordance with the ^2H NMR of Seelig and co-workers (Seelig and Seelig, 1974a). It thus appears that the observed proton and deuterium NMR order parameters arise to a large extent from significant fluctuations of the tilt of the chain as a unit about the bilayer normal, albeit on a rather long time scale (10^{-8} to 10^{-7} s). The amplitude of this chain reorientation about the bilayer normal ($\Delta\alpha \sim 50°$) is consistent with the extent of off-axis motion ($\Delta\beta \cong 55°$) previously deduced by Seiter and Chan (Seiter and Chan, 1973) in their analysis of ^1H NMR data.

The above motional model was also shown to lend itself naturally to a simple interpretation of the nuclear magnetic relaxation measurements on lipid bilayer systems. In order to account for the temperature and frequency dependences of proton T_1 data, it is necessary to invoke two correlation times of quite different time scales. In the present work, we propose that the faster time scale $\tau_\parallel$ is to be attributed to the intramolecular isomerization of the hydrocarbon chain (kink diffusion), and the slower time scale $\tau_\perp$ to cooperative fluctuations in the tilt of the chains about the bilayer normal.

The effect of surface curvature on the packing of the lipid chains in vesicle systems was also found to be readily understood in terms of the proposed motional model. The earlier line width theory of Seiter and Chan (1973) has been modified for this motional model (Hagan et al., 1977) and has been extended to the determination of deuterium line widths of rapidly tumbling vesicles. Comparison of the calculated deuterium line widths with those recently reported by Stockton et al. (1976) lead to the conclusion that introducing a high curvature in the bilayer by sonication causes significant increases in the rate and amplitude of chain tilting without appreciable disruption of the intramolecular order. This conclusion regarding the effect of sonication on the intra- and intermolecular order is consistent with the data from two recent Raman spectroscopic studies (Mendelsohn et al., 1976; Gaber and Peticolas, 1976).

The terms "fluidity" and "liquid-like" have often been used in the description of the motional state of membranes. We point out that in general it is the extent of correlation of the intermolecular interactions which determines whether a condensed phase is ordered or "liquid-like." To be sure, the conformational states of the component molecules can change upon a transition from an ordered to a disordered state, but this intramolecular effect is the consequence of the disappearance of the correlation of the intermolecular interactions rather than the reverse. For this very reason an operational definition of a liquid state is that it is that condensed state which cannot sustain shear forces. Whether on this basis a membrane should be considered "liquid-like," we feel depends on the magnitude of the order parameter S_α rather than S_γ. The evidence suggesting that S_α is much less than unity therefore indicates that the membrane is quite fluid.

The possible correlation between kink diffusion and the permeation of small molecules across lipid bilayer membranes has previously been discussed (Träuble, 1971). Whether the type of directed density fluctuations inherent to the kink diffusion mechanism does have an effect on the permeability of the membrane remains to be established experimentally. Recent studies from this laboratory, however, have led to the notion that density fluctuations can in fact generate hydrophobic ion channels which permit the translocation of even trivalent ions across the membrane (Lee and Chan, 1977).

The results obtained here also suggest biological implications for the chain reorientation motion. Clearly, membrane fluidity can be controlled by the amplitude of chain reorientation. The well-known condensation effect of cholesterol on phospholipid bilayers is readily understood in this context. A significant decrease in the angle of chain fluctuation can account for the observed increase in the order parameter by added cholesterol. Similarly, protein molecules imbedded in a bilayer membrane could by virtue of their shape or other factors modify the extent of chain reorientation of those lipid molecules in their vicinity. Such an effect can generate a structural gradient around the protein which may be felt by neighboring proteins and lead to lipid mediated protein–protein interactions. This effect is expected to be most significant at high protein/lipid ratios, where protein induced condensations of the phospholipids can produce effects similar to those observed at high concentrations of cholesterol. The condensed domains of lipids which are formed are to be distinguished from those lipids far removed from the protein molecules in terms of their motional state. They are also distinct from the boundary monolayer of lipids which has been inferred from spin-label measurements (Jost et al., 1973) since, by definition, the condensed lipids involve a much larger fraction of the total lipid pool. Protein–lipid interactions of this type are not specific with regard to the nature of the lipid and the protein, but they nevertheless can modify the overall motional properties of the membrane. An important implication of these ideas is that the activity of a specific membrane protein could be independent of the nature of neighboring proteins provided the total protein/lipid ratio is high and as long as the condensed lipid domain is present. In our judgment, such modifications of the chain reorientation

amplitude represent the most effective way of controlling the fluidity of the membrane over an extensive surface domain.

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