Minireview

Membrane NMR: a dynamic research area

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Summary

Recent NMR relaxation studies of lipid bilayers and biomembranes are explained and briefly discussed. The results of both $^2$H and $^{13}$C NMR investigations suggest that, in addition to rapid local fluctuations of the hydrocarbon chains, slower, more collective motions of the bilayer exist. When the influence of the latter is recognized and properly accounted for, the contribution from local motions can be used to estimate a value for the microviscosity of the bilayer which corresponds to that of a simple $n$-paraffinic liquid. In general, the dynamic behavior of lipid bilayers as studied by NMR appears quite similar to that of simpler liquid crystals.

Key words: NMR; lipid bilayers; liquid crystals; microviscosity; molecular dynamics.

Introduction

How can the physical properties of lipid bilayers and biomembranes be characterized and how are these structural features related to their biological function? To approach such questions, one must first inquire as to which properties are of interest, in addition to the types of experiments which should be undertaken to obtain the desired information. In fact, lipid bilayers are more akin to liquid crystals than to either the solid or liquid states, and it is this characteristic that makes them inherently distinct from the nucleic acids and the soluble and fibrous proteins whose structures have been so fruitfully investigated by means of x-ray diffraction techniques in the past. How is it, then, that one goes about describing the structural properties of lipid bilayers and biomembranes, which clearly exhibit long-range order, like a solid, yet at the molecular level are disordered and highly fluid, like a liquid?

For the case of lipid bilayers and biomembranes in the liquid crystalline state, diffraction techniques are generally limited to relatively small scattering angles, and
consequently the spatial resolution is rather low. In their detailed investigations, Luzzati and coworkers used low-angle x-ray diffraction to characterize various structural properties of lipid bilayers and derived phase diagrams for a number of lipid/water systems [1]. However, such methods do not provide information at the atomic level as a consequence of the disorder inherent in the liquid crystalline phase, which can be either static or dynamic in origin. A later advance was the introduction of $^2$H nuclear magnetic resonance (NMR) methods in conjunction with deuterium labeling by the groups of Seelig and others in the 1970s. The information obtained from $^2$H NMR studies can be related to the polar head group conformation, the mean bilayer thickness, and various thermodynamic quantities [2–5]. Nevertheless, both x-ray diffraction and $^3$H NMR lineshape studies have a common feature in that the experimental results chiefly involve average properties of the bilayer – since information on both the segmental ordering and dynamics is needed, such studies provide only a part of the story.

It is here that the versatility of NMR techniques can be employed to great advantage. In fact, NMR is practically unique among the common biophysical methods in providing information related to the above quantities without introduction of probe molecules into the bilayer, which may alter the structural properties of interest. Among the most interesting and novel of the multifaceted NMR methods are the various relaxation time measurements. These powerful NMR techniques are directly analogous to other, perhaps more familiar relaxation methods. Taken together, an analysis of the $^2$H NMR spectra and the NMR relaxation times of lipid bilayers can provide the basis for a more complete description of their structural properties than has been possible in the past. The NMR lineshapes provide information on the segmental ordering, which is related to the averaged molecular structure, whereas an analysis of the relaxation behavior can provide information on molecular motions at the segmental level. What can such NMR relaxation methods tell us about the dynamics of a lipid bilayer or biomembrane?

The hydrocarbon region of a lipid bilayer

We need first to briefly discuss the results of $^2$H NMR studies of the model 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayer [2,3,6–8]. As mentioned above, one primary aspect of biophysical studies of membranes is to define and measure experimental observables related to the ordering and dynamics of the fatty acyl chain segments which comprise the interior hydrocarbon region of the bilayer. By analyzing the $^2$H NMR spectral lineshapes of lipid bilayers in the liquid crystalline (L$_c$) phase, one can obtain a simple and unambiguous measure of the average structure in terms of what is called the order parameter of the various $^2$H-labeled bond segments, $S_{CD}$, defined [2,3,8] as

$$S_{CD} = \frac{1}{2} \langle 3 \cos^2 \beta(t) - 1 \rangle.$$

In the above, $\beta(t)$ is the angle between the C-$^2$H bond direction at any given instant
Fig. 1. Schematic illustration of a lipid bilayer indicating the angle between the carbon–deuterium (D) bond direction at any given instant and the perpendicular to the membrane surface \( n_0 \). The average orientation of a given deuterium labeled segment can be described in terms of the order parameter \( S_{CD} \) (see text).

and the perpendicular to the membrane surface (cf. Fig. 1), and the brackets denote an average over all possible bond orientations on the \(^2\text{H} \) NMR time scale \(( < 10^{-5} \text{ s})\).

A précis of current experimental results is included in Fig. 2, which depicts profiles of the order parameters, \( S_{CD} \), determined from \(^2\text{H} \) NMR studies of DPPC bilayers specifically deuterated in the fatty acyl chains, plotted as a function of the

Fig. 2. Summary of \(^2\text{H} \) NMR studies of DPPC in the liquid crystalline (\( L_a \)) phase [2,3,7]. Data are indicated for multilamellar dispersions of DPPC specifically labeled with \(^2\text{H} \) at a particular methylene segment of both fatty acyl chains at 51°C [7]. The order parameter \( S_{CD} \) (○) and the spin-lattice relaxation rate \( T_1^{-1} \) (●) are plotted as a function of the deuterated segment position.

Fig. 2. Schematic illustration of a lipid bilayer indicating the angle between the carbon–deuterium (D)
labeled methylene segment position. Similar profiles of the $^2$H spin-lattice relaxation rate, denoted by $T_1^{-1}$, are also shown in Fig. 2 and will be discussed shortly. The order parameter profile represents a trademark or characteristic 'signature' of the fluid, liquid crystalline state. $S_{CD}$ is roughly constant in absolute value and equal to about 0.2 over the initial portion of the chains, with a progressive decrease for those chain end segments near the bilayer central region. A simple explanation of the order parameter is the following: If the phospholipid fatty acyl chains were in the all-trans configuration, and the molecules were rotating about their long axes, then an order parameter of $|S_{CD}| = 0.5$ would be expected (cf. Eqn. (1) with $\beta = 90^\circ$). Isotropic motion over all space, on the other hand, would lead to $|S_{CD}| = 0$. Thus, we can immediately conclude that the phospholipid molecules exist in a state of intermediate order since the $|S_{CD}|$ values lie between these two extremes. The possible sources of the disorder could include trans-gauche isomerizations of the phospholipid fatty acyl chains as well as 'tilting' motions of whole molecules or parts of the molecules within the bilayer.

How can we explain the shape of the order profile, namely the observation of a 'plateau' with a decrease in $|S_{CD}|$ near the bilayer center? The fact that a plateau is observed for those segments closest to the aqueous interface suggests that coupled gauche $\leftrightarrow$-trans-gauche $\leftrightarrow$ isomerizations are favored, which leave those chain segments before or after the defect aligned perpendicularly to the membrane surface as in the all-trans chain conformation. The presence of such relatively small structural defects as 'kinks' and 'jogs' then leads to a largely parallel packing of the chains in the bilayer hydrocarbon interior [2]. Since the presence of gauche isomers results in a shortening of the effective fatty acyl chain length projected along an axis perpendicular to the bilayer surface, clearly a statistical distribution of effective chain lengths will be present. To maintain the density of the bilayer hydrocarbon region approximately constant, those end segments adjacent to the chains with the shorter effective lengths at any given instant must be more disordered to reduce the free volume, thereby explaining the lower $|S_{CD}|$ values near the bilayer center [9,10]. The order profiles, which correspond to microscopic, segmental properties of the membranous phospholipids, can be further interpreted in terms of the average bilayer thickness and other, macroscopic thermodynamic properties [2,11].

But what of the bilayer dynamics? Information regarding the nature of the molecular motions which lead to the observed $S_{CD}$ values and their rates can be obtained from an analysis of the NMR spin-lattice ($T_1$) relaxation times. As shown in Fig. 2, the profile of the spin-lattice relaxation rate $T_1^{-1}$ as a function of the deuterated chain segment position is qualitatively similar to the order profile. That is, an approximate 'plateau' is observed, followed by a decrease in the central region of the bilayer. Since the order profile, obviously, depends only on the ordering, whereas the $T_1^{-1}$ profile depends on both the amplitudes and the rates of the motions of the fatty acyl chain segments in the bilayer hydrocarbon interior, how are the order and $T_1^{-1}$ profiles related? Moreover, how does one go about separating the static and dynamic information contained in the latter? Before we delve into these problems, we need to further inquire into the process of spin-lattice relaxation and how it is measured.
NMR relaxation

Basically, NMR relaxation times are measured by what can be thought of as a 'magnetization-jump' experiment - the spectral magnetization is first perturbed away from equilibrium by a suitable radiofrequency pulse scheme, and then it is followed as a function of time until equilibrium is restored. The time constant for the first-order recovery of the longitudinal magnetization (i.e., along the applied magnetic field direction) is called $T_1$, the spin-lattice relaxation time. How are such spin-lattice or $T_1$ relaxation studies related to the dynamic properties of membranes? Briefly stated, the interpretation of NMR relaxation experiments, with the attendant physical implications, requires the introduction of a suitable theoretical paradigm. Thus, the desired information is always extracted in a model-dependent manner, and we wish then to ask how our simple theoretical pictures match up with the available experimental results. The reader should recognize that no model is unique and that several alternative interpretations may be possible.

Lipid bilayers and paraffinic liquids

As a simplified first approach, let us assume that the hydrocarbon region of a bilayer composed of a typical phospholipid such as DPPC is similar in its local dynamics to the corresponding liquid paraffin, such as n-hexadecane, where both systems are assumed to be above their chain melting temperatures. One might imagine that the lateral bilayer pressure due to short-range, repulsive intermolecular interactions [2,9-11] could introduce significant correlations in the chain fluctuations arising from isomerizations, torsional oscillations, etc., which would most likely maintain the parallel chain packing in the bilayer as discussed above. However, insofar as the rates of local motions of the bilayer hydrocarbon chain segments are concerned, why should these be greatly different from the corresponding n-alkanes? Therefore, let us assume for a start that they are similar, and see where this leads us.

Now, the reader will recall that the spin-lattice relaxation time $T_1$ is related to the lifetime of a given nucleus in its various spin energy levels, which as mentioned earlier depends on the rates and amplitudes of the molecular motions. Denoting the contribution to the overall spin-lattice relaxation rate from rapid, local motions as in n-alkanes by $T_{1f}^{-1}$, the standard NMR theories yield that

$$1/T_{1f} = A \tau_t,$$

where $A$ is a constant and $\tau_t$ is the correlation time for these fast or local-type motions (like a 'memory-time'). However, the above equation assumes that the correlation time $\tau_t$ for the rapid local motions is less than $1/\omega_0$, where $\omega_0$ is the NMR resonance frequency (the so-called 'fast motional' or 'short correlation time' limit). This implies that (i) $T_1$ should increase with temperature, and (ii) $T_1$ should be independent of the resonance frequency $\omega_0$ (related to the magnetic field strength, $B_0$ by $\omega_0 = \gamma B_0$, where $\gamma$ is a constant). For the case of n-alkanes we know that this is true [12,13]. But what about for lipid bilayers?
A dilemma

It turns out for lipid bilayers [7] and also for native biomembranes [14], that although $T_1$ does increase with temperature as for the $n$-alkanes, the $T_1$ times also exhibit a significant frequency dependence [15,16]. This frequency dependence is a major feature of the relaxation, which must be accounted for in addition to the dependence of $T_1$ on temperature and on the degree of segmental ordering in the bilayer. For example, consider the case where $T_1$ measurements were made at a single magnetic field strength, that is resonance frequency, and then Eqn. (2) was applied to extract a correlation time $\tau_\phi$ for a given hydrocarbon chain segment. The value of $\tau_\phi$ could then be employed to calculate the local or segmental microviscosity of the bilayer from the equation $\tau_\phi = V_n/kT$, where $V$ is the segmental volume and $\eta$ the microviscosity. However, if $T_1$ measurements were made at yet another magnetic field strength or the results using different nuclei with different resonance frequencies were compared, e.g. $^2$H and $^{13}$C, one would obtain different values for $\tau_\phi$ using Eqn. (2). Thus the calculated microviscosity $\eta$ would depend on the magnetic field strength, i.e. resonance frequency, as well as the nucleus employed.

Lipid bilayers and liquid crystals

How do we extricate ourselves from this dilemma? As a working hypothesis, let us now assume that in addition to fast or local-type segmental motions as discussed above, there exist in lipid bilayers an additional class of motions of larger amplitude. These slower motions would account for the observed relaxation enhancement in bilayers compared to $n$-alkanes ($T_1$ is shorter in the former), as well as the observed frequency dependence of the relaxation. In addition, let us assume that the slow motions of larger amplitude in bilayers correspond to the types of relatively slow motions postulated for liquid crystals. In liquid crystalline parlance such motions are termed ‘director fluctuations’ or ‘order fluctuations’. Representative examples are schematically depicted in Fig. 3. Cooperative, whole molecule motions of the type illustrated would require less energy than tilting of the molecules independently as rigid rods, and could occur via thermal fluctuations in the modes or by phospholipid lateral diffusion. The key idea is that one has a continuous distribution of wave-like disturbances which undergo viscous or first-order relaxation, each with a characteristic amplitude, wavelength and correlation time. In the frequency domain, the continuous distribution of correlation times leads to a predicted $T_1^{-1}$ dependence which goes as $\omega_0^{-1/2}$. Since both the fast and slow motions are assumed to be axially symmetric about the bilayer normal, the collective-type slow motions will then be described mathematically by a relaxation contribution $T_{1s}^{-1}$ of the form [15–18]

$$1/T_{1s} = BS_{CD}^2 \omega_0^{-1/2},$$

where $S_{CD} (= S_{CH})$ is the bond segmental order parameter. In the above equation, $B$ is a collection of constants that will not further concern us here. The slow term $T_{1s}^{-1}$
accounts for the dependence of the relaxation rate on frequency \( (\omega_0) \) and ordering \( (S_{CD}) \), whereas the temperature dependence could in principle arise from either or both of the \( T_{1f}^{-1} \) and \( T_{1s}^{-1} \) contributions [16,17]. Thus, we simply assume that each of the segments in the bilayer hydrocarbon region behaves analogously to a rod-like molecule in a nematic fluid. While there is certainly no a priori reason why this has to be so, the idea is attractive enough to warrant further examination.

Given the above, one would then write for the experimentally measured relaxation rate that \( T_1^{-1} = T_{1f}^{-1} + T_{1s}^{-1} \), where the first and second terms on the right are given by Eqns. (2) and (3). Physically we would imagine that the rapid local motions in the bilayer hydrocarbon region would occur at about the same rate as in liquid \( n \)-paraffins, but the fact that the lipid molecules are effectively ‘attached’ to the aqueous interface by their head groups together with the repulsive interactions between the chains [9–11] would then lead to restrictions in the amplitude of their rapid segmental motions [2,3,6,7]. The local ordering set up by the fast motions [6,7] could then be averaged by any slower, collective-type fluctuations of the type discussed above [15–19]. That is, the part of the relevant magnetic interactions of interest which is ‘left-over’ from the fast motions, e.g., as a consequence of the local ordering, is further modulated by the slower motions. Thus, the concept of different motional ‘time-scales’ is quite naturally built into our theoretical paradigm.

**Evidence from NMR**

What grounds exist for such a model of the bilayer dynamics? Suffice it to say here that such a simple, perhaps naive, picture does appear to hold up at present for
Fig. 4. Experimentally measured dependence of the spin-lattice relaxation rates of the DPPC acyl chain segments, denoted by $T_1^{-1}$, on the degree of segmental ordering ($S_{CD}$) and resonance frequency ($\omega_0$). (a) The $^2$H $T_1^{-1}$ rates are plotted versus $(S_{CD})^2$ as determined from $^2$H NMR studies of specifically labeled DPPC multilamellar dispersions [7]. (b) The corresponding $^{13}$C $T_1^{-1}$ rates versus $\omega_0^{-1/2}$ determined from studies of unilamellar DPPC vesicles [16,17].

The contribution from fast, local-type motions can be obtained from the $y$-intercept of either of the plots in Fig. 4; a value of $\tau_i \approx 10^{-11}$ s is obtained [16,17]. This is a very small number – about an order of magnitude less than previous estimates [3,7,20]. However, we note after extrapolating away the frequency dependence of $T_1^{-1}$ for the DPPC bilayer, that the $T_{1f}^{-1}$ contribution is in good agreement with the
frequency-independent $T_1^{-1}$ values for $n$-hexadecane, as is shown in Fig. 4b. This result suggests that the microviscosities of the two systems are very similar. That is, the microviscosity experienced by the acyl chain segments of the DPPC bilayer hydrocarbon region, for which a bulk viscosity cannot be measured, would appear roughly the same as that of a liquid $n$-paraffin having a bulk viscosity of about 1–2 centipoise. (The above microviscosity should be contrasted with the ‘effective membrane viscosity’ derived from studies of the rotational diffusion of proteins such as rhodopsin [21,22].) Thus the analysis has come full circle – we started by considering the segmental motions of bilayers in terms of the corresponding $n$-al-kanes and then asked what additional features needed to be incorporated to account for the $T_1$ data. The end results of the analysis illustrate the dynamic similarities of the two systems, as well as distinguish the characteristic properties of the liquid crystalline state of a lipid bilayer from those of the simpler liquid $n$-paraffins.

Further comments on the bilayer microviscosity

The above can be restated somewhat differently as follows: by extrapolating away the $T_1$ frequency dependence for the DPPC bilayer in a model-dependent fashion, one can conclude that the local motions occur at about the same rate as in liquid $n$-paraffins of similar chain length. If $T_1$ measurements were made at a single magnetic field strength, one would find that $T_1$ was significantly shorter for DPPC compared to $n$-hexadecane, and at present we could never find a high enough magnetic field strength to completely eliminate the $T_1$ contribution from slow motions in the former. Thus, if measurements were made at only one frequency [3,20], we would always conclude that the microviscosity of the bilayer chain segments corresponded to a slower rate of local motions relative to $n$-alkanes. But, once the existence and nature of the $T_1$ frequency dependence is recognized, it is simple to deduce that the rate of the local motions is probably quite similar in the two systems, and that the relaxation enhancement together with the frequency dependence in bilayers can be explained in terms of their added liquid crystalline features [16,17].

Relation to fluorescence studies

The above conclusion, namely that the ‘fluidity’ of the hydrocarbon region of a lipid bilayer matches rather closely that of a liquid $n$-paraffin, appears to be at odds with some of the earlier fluorescent probe studies of membranes [23]. As discussed elsewhere [24–27], there may be problems with the interpretation of previous fluorescence studies which are only now being resolved. Perhaps foremost is that the probe dynamics may not accurately manifest the dynamics of the host bilayer, but it should also be pointed out that the calculated microviscosity values are highly dependent on the probe and reference solvent employed [24]. Furthermore, the interpretation of the rotational relaxation rates of fluorescent probes in terms of the
bilayer microviscosity may not be quite as simple as assumed in the past [26–30]. For example, it can be shown [6,27] that the fluorescence anisotropy ratio $r_\infty/r_0 = S^2$, where $S$ now denotes the order parameter of the fluorophore emission dipole, so that both the time-resolved and steady-state anisotropy depend on the degree of probe ordering in addition to the correlation time for reorientation [26,27,30]. The correspondence of fluorescence depolarization studies utilizing probes such as parinaric acid to the NMR results summarized in Fig. 4 is obviously of some interest. Hopefully, one can soon expect a convergence of the NMR [16,17] and fluorescence [28] points of view.

Conclusions and future outlook

In the roughly 15 years since the earliest applications of NMR relaxation methods to lipid bilayers our knowledge has increased steadily. At the present time, we can begin to propose specific models for the molecular dynamics of these systems and to test them experimentally. In the future, one would like to carry out studies of different lipid bilayers and to further investigate the possible influences of cholesterol and integral membrane proteins such as rhodopsin or bacteriorhodopsin on the bilayer structural dynamics. Given the biological importance of cooperative phenomena, greater emphasis should probably be placed on applications of NMR methods sensitive to lower frequency motions of lipid bilayers and biomembranes. Thus, $T_1$ measurements at low magnetic field strengths, rather than the currently popular high field strengths, are anticipated to be valuable, in addition to rotating-frame ($T_{1p}$) studies, which correspond to relaxation in the much smaller oscillating magnetic fields used to detect NMR. Recently, the first applications of high-resolution, solid-state $^{13}$C NMR methods to lipid bilayers and biomembranes have been reported [31]. In reconstituted membranes, the presence of rhodopsin, the visual pigment, leads to an approximately two-fold reduction in the $T_{1p}$ values of the phospholipid resonances, indicating a substantial influence on molecular motions. Given the continued rapid pace of development of new techniques, the future appears bright for the application of NMR methods to studies of membrane structural properties – clearly a dynamic area of research!

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