Smoothed orientational order profile of lipid bilayers by ^{2}H-nuclear magnetic resonance

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ABSTRACT A new method has been developed to determine the complete orientational order profile of lipid bilayers using ^{2}H-NMR. The profile is obtained from a single powder spectrum of a lipid which has a saturated chain fully deuteriated. The smoothed order profile is determined directly from the normalized dePaked spectrum assuming a monotonic decrease of the order along the acyl chain. The oscillatory variations of the order at the beginning of the chain are not described by this method. However the smoothed order profile reveals in a straightforward way the crucial features of the anisotropic order of the bilayer.

INTRODUCTION

In biological membranes, the lipid bilayer is generally in the liquid-crystalline (L_α) phase (Singer and Nicholson, 1972) where the axis of symmetry for the motions of the acyl chains is perpendicular to the plane formed by the polar head groups. This macroorganization of the lipids imposes a structure on the acyl chains where the orientational order has been found to vary in a charateristic manner along the chains, being largest for the methylene groups in the portion closer to the lipid-water interface and decreasing rapidly near the tail.

Deuterium nuclear magnetic resonance (^{2}H-NMR) spectroscopy has proven to be an important technique for the characterization of the L_α phase (Mantsch et al., 1977; Seelig and Seelig, 1980; Davis, 1983) because the ^{2}H-NMR quadrupolar splitting is proportional to the local orientational order parameter, $S_{CD}(n)$, defined by:

$$S_{CD}(n) = \frac{1}{2}\langle 3\cos^2\theta_n - 1\rangle \quad (1)$$

where θ_n is the angle between the C—D bond for the nth carbon position and the axis of symmetry of the rapid motions of the acyl chain. The angular brackets represent an average over molecular conformation and orientation on the NMR timescale.

The first ^{2}H-NMR experiments to determine the dependence of $S_{CD}(n)$ on the carbon position were achieved using specifically labeled lipids (Seelig and Seelig, 1977 and 1980). The orientational order profile along the chains was described by a set of discrete values corresponding to specific carbon positions on the chains. This method, though it gives an accurate and detailed description of the order profile, requires a substantial investment of time and effort because each value of the local order requires the synthesis of a specifically labeled lipid and the acquisition of a separate spectrum.

A second method has been proposed to determine the shape the orientational order gradient in bilayers using moments of deuterium magnetic resonance spectra of a sample containing lipids with perdeuteriated chains (Bloom et al., 1978; Davis et al., 1980). Because the deuterium nuclei are uniformly distributed along the chain in such a sample, the signal obtained contains information about the complete order profile. This second method was based on the relationship between the moments of the powder spectra and the moments of the orientational order distribution along the chain. This distribution was assumed to be well approximated by an empirical function such as:

$$S(x) = S(0) \times (1 - \mu x^\nu), \quad (2)$$

where $S(0)$, μ, and ν were adjustable parameters and x a continuous variable associated with the chain position. The moments of the spectrum were expressed in terms of the adjustable parameters which were determined from a least squares fit of the experimental values of the first four moments (Bloom et al., 1978; Davis et al., 1980); the profile was then described by the continuous function $S(x)$.

The order profile of the L_α phase has been also obtained from dePaked ^{2}H-NMR spectra of lipids with a single perdeuteriated chain (Paddy et al., 1985; Pauls et al., 1983). The quadrupolar splittings were measured directly on the dePaked spectra for the resolved doublets and a constant value of S measured from the unresolved doublets was assigned to the plateau segment. The profile was described by this set of discrete values.

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In this paper, a new method to determine the orientational order profile is presented. This method, in common with the moments analysis method, uses phospholipids with perdeuteriated acyl chains but does not assume a shape of the order parameter distribution. The smoothed order profile is obtained directly from the dePaked spectra assuming a monotonically decreasing variation of $S(n)$ with n. The method has previously been applied in an empirical way to compare the order parameter profiles in the L_α and H_{II} phases (Sternin et al., 1988). Here we compare the results of this method for POPC with those obtained using specific labels to demonstrate the general application of the method.

MATERIALS AND METHODS

POPC-d_{31} (1-palmitoyl-2-oleoyl-phosphatidylcholine, where the palmitoyl chain is perdeuteriated) was obtained from Avanti Polar Lipids Inc. (Birmingham, AL). The lipid showed a single spot on TLC and chain analysis indicated an equimolar mixture of palmitoyl and oleoyl chains. The lipid dispersion was prepared in Hepes buffer 20 mM, 300 mM NaCl, pH 7.4, made in deuterium depleted water (Sigma Chemical Co., St. Louis, MO).

The deuterium NMR spectrum was obtained on a home-built 46 MHz ^{2}H-NMR spectrometer described in detail elsewhere (Davis, 1979; Sternin, 1985). The spectrum was collected for 100,000 transients using a quadrupolar echo sequence and phase cycling (Davis, 1979 and 1983; Rance and Byrd, 1983). The 90° pulse length was 4 μs and the time between the two 90° pulses in the quadrupolar echo pulse sequence was 50 μs. 30 μs after the second pulse, 2,048 points were collected in quadrature with a dwell time of 5 μs. Successive pulse sequences were separated by 300 ms. The temperature of the sample was regulated by a model BV-T1000 temperature controller (Bruker Instruments, Inc., Billerica, MA).

RESULTS AND DISCUSSION

The powder spectrum of a dispersion of POPC-d_{31} in the fluid phase (Fig. 1) is complex because it involves contributions from every position along the perdeuteriated palmitoyl chain and all orientations of the bilayer normal relative to the magnetic field. Actually the deuterium nuclei of every carbon position have a distinct order parameter, so that the spectrum is a superposition of 15 different powder spectra, and it is difficult in this case to assign specific splittings from the powder spectrum. Assuming that the interactions responsible for the lineshape scale as $P_2(\cos\theta)$, it is possible to convert the powder spectrum into one which is characteristic of an oriented sample using the dePakeing procedure (Bloom et al., 1981; Sternin et al., 1983). The resulting dePaked spectrum for an orientation of 0° is shown in Fig. 1 b. It shows directly the continuous distribution of order parameters. In the dePaked spectrum shown in Fig. 1, eight doublets are resolved. The remaining seven doublets[1] are associated with the most intense peak which has the largest quadrupolar splitting. This portion of the spectrum is associated with the plateau region of the profile (Davis, 1983). To determine the orientational order gradient across the bilayer, this distribution of order parameters is expressed in terms of an average order for every labeled position along the chain. First, the well-resolved innermost doublet is assigned directly to the terminal methyl because its relative area corresponds to three deuterium nuclei and the methyls are the most mobile labeled groups. The remaining area of the dePaked spectrum is normalized to the 28 deuterium nuclei of the methylene groups contributing to the signal and divided into areas associated with the 14 carbon positions of the palmitoyl chains. A mean order parameter is then calculated for each region.

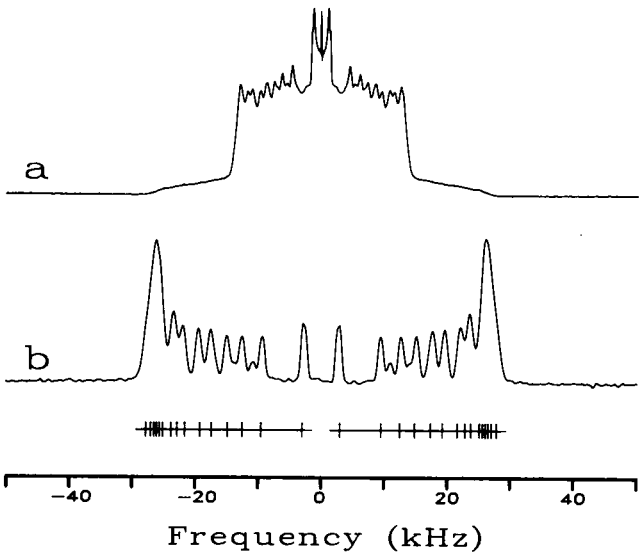

FIGURE 1 ^{2}H-NMR spectrum of POPC-d_{31} at 27°C. The powder spectrum (*a*) exhibits the normal lineshape for a phospholipid-water dispersion in the lamellar (L_α) phase. The resulting dePaked spectrum (*b*) can be used to determine the distribution of the quadrupolar splittings as described in the text. The tick marks represent the middle of an area associated with one CH_2 group, except for the innermost ones assigned to the terminal methyl.

If it is assumed that the orientational order in POPC decreases monotonically along the chain from the interface towards the middle of the bilayer, the order parame-

[1]Some comment should be made about the small peak corresponding to the third smallest quadrupolar splitting. We associate it with a fraction of lipid molecules in which the deuteriated chain has transmigrated to the *sn*2 position during the chemical preparation. Previous studies have shown that for lipids, the same carbon position on the *sn*1 and *sn*2 chains are not equivalent (Seelig and Seelig, 1980; Paddy et al., 1985). The fractional area of the smaller peaks indicates that the transmigration is ~ 20%.

ters shown in Fig. 2 are obtained. These values are compared with results previously determined using specifically labeled POPCs (Seelig and Seelig, 1977). As one can see, the profile obtained by this integration method agrees well with that obtained using 11 specifically labeled PCs and describes the main features of the bilayer signature: near the interface, the order parameter varies gradually with the carbon position defining a plateau in the $S(n)$ vs. n plot. This plateau is followed by a rapid decrease of the order towards the middle of the bilayer.

Closer examination shows that the assignment made for the plateau region contains systematic errors. This is due to the fact that the assumption of a monotonic decrease is not valid for this part of the chain. There are local oscillatory variations of $S(n)$ vs. n in the first few carbon positions (Seelig and Seelig, 1980) reflecting conformational restrictions imposed by the geometry of the lipid. The method presented here does not give this detailed local structure of the plateau but does reveal the general shape of the flexibility gradient. We believe that the crucial physical information required to characterize the anisotropic order of membranes is contained in the smoothed shape of the $S(n)$ vs. n plot rather than in the local variations so that is not necessary, in most cases, to use specifically labeled lipids in ^{2}H-NMR studies of the order profile.

To demonstrate that the results extracted from the dePaked spectrum describe the orientational order distribution encoded in the powder spectrum, we calculated the moments of the spectrum from the order profile: this is the inverse of the method described in Bloom et al. (1978) and Davis et al. (1980). First the order profile is characterized by a continuous function. We chose to fit a very similar function to that assumed previously to describe the profile from the moments of the powder spectrum (Bloom et al., 1978; Davis et al., 1980). The position along the chain is normalized between 0, the beginning of the chain, and 1, its end, using

$$x = \frac{n - n_0}{16 - n_0}. \tag{3}$$

In the fit, the index n_0, the beginning of the chain, is an adjustable parameter whereas the end is taken as the position 16. It is necessary to correct the absolute value of the order parameter at this position since the different symmetry of the terminal methyl affects the order parameter. The S(16) value has been linearly extrapolated from S(14) and S(15). The function used to describe the profile is

$$S(x) = S(0) \times \left[1 - \left(1 - \frac{S(16)}{S(0)}\right)x^{\nu}\right], \tag{4}$$

and n_0, $S(0)$, and ν are the three adjustable parameters. The continuous distribution obtained by a least-square fit is represented by the curve in Fig. 2. The values of the adjustable parameters are $S(0) = 0.216$, $n_0 = -0.630$, and $\nu = 2.642$. The value of $n_0 = -0.630$ that we obtained

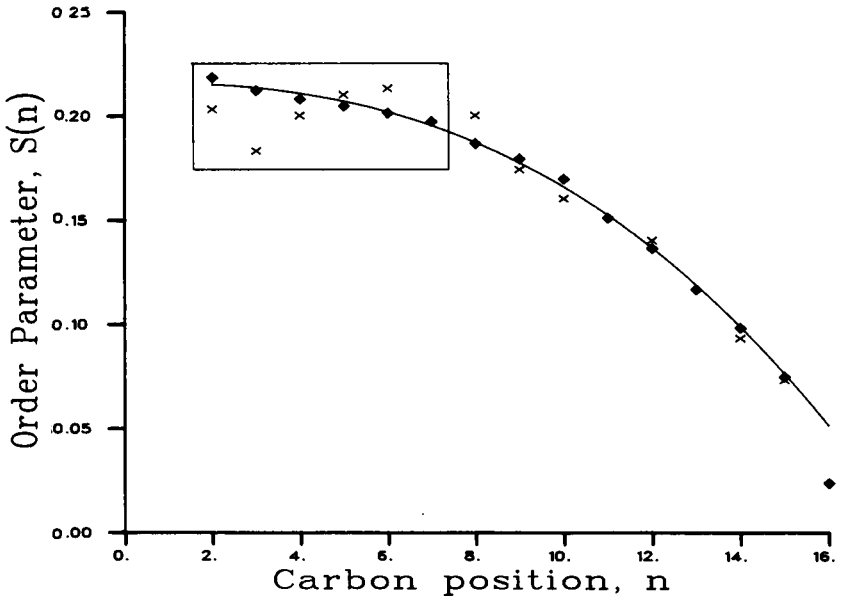

FIGURE 2 Order profile of POPC at 27°C. The order profile derived from the powder pattern of POPC-d_{31} as described in the text is represented by ♦. The curve represents the function obtained by the least squares fit. The order profile determined by selectively labeling carbon positions (Seelig and Seelig, 1977) is presented for comparison (represented by +). The box indicates the plateau region.

corresponds roughly to the carbon atom C_1 of the glycerol. This is very reasonable because n_0 represents in the fit the beginning of the chain and its value corresponds with the location of the point of attachment of the *sn*1 chain to the glycerol backbone.

The moments can be expressed in terms of the moments of the distribution of the order parameters, according to

$$M_r = A_r\left(\frac{3}{4}\frac{e^2qQ}{\hbar}\right)^r S_r \qquad (5)$$

where A_r is a constant derived from the expression for the quadrupolar powder pattern lineshape function, $e^2qQ/\hbar$ is the quadrupolar coupling constant (167 kHz for a C—D bond). It has been calculated elsewhere from the quadrupolar powder pattern lineshape function (Davis et al., 1980) that $A_1 = 2/3\sqrt{3}$, $A_2 = 1/5$, $A_3 = 2/35\,(1 + 2\sqrt{3})$, and $A_4 = 3/35$. Assuming that the deuteriums are distributed uniformly all along the chain, the expression of the moments of the distribution of the order parameters is then given by

$$S_r = \frac{1}{x_{max} - x_{min}}\int_{x_{min}}^{x_{max}} [S(x)]^r\,dx. \qquad (6)$$

This integration has been made numerically, integrating between $n = 2$–16, the part of the chain bearing deuterium nuclei. The comparison between the experimental moments and the calculated values is presented in Table 1. As one can see, the agreement for the low moments ($r = 1$–3) is good, reinforcing the validity of the method. The calculated moments are slightly higher than the experimental values because the approximation of uniformly distributed deuteriums underestimates the contribution of the methyl groups and because the correction of the $S(16)$ increases again the calculated value of the moments over the measured ones. We estimate the systematic error in the evaluation of the moments due to these factors to be ~10%, which is the approximate discrepancy with the measured moments.

CONCLUDING REMARKS

The new method presented in this paper determines the smoothed order profile of a lipid with a perdeuteriated saturated chain, based on the continuous distribution of order parameter explicitly described by the dePaked spectrum. Even though this concept is considerably different from the one describing the profile by a set of discrete order parameters measured on the dePaked spectra (Paddy et al., 1985; Pauls et al., 1983), both methods lead to very similar results for profiles of the L_α phase. The discrete approach, however, is influenced by the number of resolved doublets. In the H_{II} phase for which none of the methylene groups are resolved in the dePaked spectra (Sternin et al., 1988), the discrete method cannot be used, whereas the order profile can be described using integration method. Because there is no preliminary assumption on the profile shape, the integration method is also more versatile than the method using the moments. The very good agreement with the profile obtained with specifically labeled lipids shows that this new method describes the general features of the order gradient accurately, whereas the use of perdeuteriated chains reduces considerably the problems of synthesis and data acquisition related to the use of specific labels. The fine structure of the profile, such as the variations observed for the plateau region, cannot be determined by this method and should be measured with local probes. We believe that these local variations reflect local geometry of the acyl chains and are not of primary interest in characterizing the anisotropic nature of bilayer order. Several theories (Marčelja, 1974; Jähnig, 1979; Dill and Flory, 1980; Meraldi and Schlitter, 1981) have reproduced the order distribution along the chain. Two of them (Jähnig, 1979; Dill and Flory, 1980) give smoothed profiles. To simulate accurately the experimental data, the statistical mechanical treatment (Meraldi and Schlitter, 1981) had to introduce several free parameters to express the geometrical properties of chain conformations. However, in the Meraldi and Schlitter (1981) model as well as in the others, the overall shape of the order profile is reproduced taking in account the main factors responsible for the organization of the lipids such as *trans-gauche* isomerization, lateral pressure, van der Waals interactions, and steric hindrance. The method presented in this paper constitutes a straightforward and efficient experimental method of studying changes in the crucial features of lipid organization by the systematic variation of factors which modulate order in anisotropic structures.

TABLE 1 Comparison between the experimental values of moments and the calculated values from orientational order profile

	M_1	M_2	M_3	M_4
	s^{-1}	s^{-2}	s^{-3}	s^{-4}
Experimental values	4.65×10^4	3.26×10^9	2.84×10^{14}	2.29×10^{19}
Calculated values	4.90×10^4	3.54×10^9	3.17×10^{14}	3.32×10^{19}

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