Information on Bond Orientation Distributions in Lipids and Liquid Crystals from Segmental Order Parameters

K. Schmidt-Rohr

Department of Polymer Science and Engineering, University of Massachusetts, Amherst, Massachusetts 01003

M. Hong*

Materials Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, and Department of Chemistry, University of California, Berkeley, California 94720

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The information content of segmental order parameters in liquid-crystalline lipids is analyzed in terms of orientation distributions. First, we discuss the use of segmental order tensors, determined from motionally averaged NMR anisotropies, for obtaining approximations to the orientation distribution of the director in the molecular segment. In addition to the distribution consisting of second-order spherical harmonics, two physically reasonable five-parameter distributions are examined, and the relation of the two shape parameters of these distributions to the principal order parameters is presented. The usefulness of these distributions for the determination of torsional angles is also discussed. Furthermore, we introduce a novel approach for obtaining model-independent limits to bond orientation probabilities from bond order parameters. The analysis shows, for instance, that the C−H bonds of the glycerol *sn*-3 CH$_2$ group in phosphatidylcholine, with bond order parameters of −0.22, are oriented preferentially at angles between 52° and 128° from the director. The probability that these bonds are oriented at angles smaller than 30° or larger than 150° is less than 25%. Assuming an upper limit to the ruggedness of the distribution, even more stringent probability limits can be given. For instance, using a minimum standard deviation of 10° for any conformation, a C−H order parameter of −0.22 corresponds to a probability of less than 25% for C−H orientations below 51° and above 129°. The approaches described here are generally applicable to uniaxial liquid-crystalline systems.

Introduction

The investigation of the conformational structure of lipids in the liquid-crystalline L$_\alpha$ phase is important for a detailed understanding of the function of these molecules in biomembranes.[1−3] Various scattering and spectroscopic techniques have been applied to elucidate the conformations of hydrated lipids.[4−10] However, the mobility and flexibility of these molecules render the interpretation of the data difficult, since many interchanging conformations must be considered.[11] For phospholipids, the most important model-independent information on the molecular conformation has been provided by neutron scattering, which showed that the acyl chains and the glycerol backbone are roughly parallel to the bilayer normal, while the headgroup is nearly parallel to the bilayer surface.[4] In addition, ^{2}H NMR has shown that the headgroup interchanges fast between at least two symmetry-related conformations.[7]

NMR, with its ability to distinguish individual chemical moieties, has a great potential for analyzing the orientations of individual segments in lipid molecules relative to the local director. In this paper, we present two complementary approaches for extracting structural information from NMR data, with few or no model assumptions. In the first approach, we discuss how segmental order tensors can yield approximations to the orientation distributions of the segments relative to the director and how they may be useful for determining molecular conformations. The second approach provides a model-independent conversion of bond order parameters into well-defined probabilities of segmental orientations. Its potential is demonstrated for phosphocholine, where the signs and magni-

tudes of C−H bond order parameters have recently been determined for most sites.[12]

Orientation Distributions and Order Tensors

Due to the flexibility of lipid molecules, a model showing a single structure probably provides only a poor approximation to the actual dynamic molecular conformation. Therefore, more realistic descriptions of the lipid structure need to incorporate many more parameters. An analysis in terms of probability distributions of torsional angles or of internuclear vector orientations seems to be particularly promising.[13] In uniaxial liquid-crystalline systems, information on the distribution of the director in each segment of the molecule is accessible by NMR spectroscopy.

1. NMR Determination of Segmental Order Tensors. The most complete analysis of motionally averaged, segment-fixed NMR interactions can be given in terms of segmental order matrices.[1,9,14,15] For a molecule that undergoes uniaxial motions with respect to the director (macroscopic uniaxiality), the order matrix of each segment in any segment-fixed frame is given by

$$S_{jk} = {}^1\!/_2 \langle 3 \cos \gamma_j \cos \gamma_k - \delta_{jk} \rangle \quad (1)$$

where γ_j is the angle between the director and the j axis of the segment-fixed frame. By this definition, the order matrix is traceless ($S_{11} + S_{22} + S_{33} = 0$) and symmetric ($S_{jk} = S_{kj}$) and thus contains five independent elements. If in addition to the macroscopic uniaxiality the molecule also possesses microscopic uniaxiality as a result of, for example, rotation around a molecular axis, then the order tensor is uniaxial, and its largest

principal value, $S_{\parallel}$, is the conventional order parameter. $S_{\parallel}$ is unity for perfectly aligned molecules and zero for an isotropic system. In systems without microscopic uniaxiality, the largest principal value of the segmental order matrix provides a lower limit to the overall molecular order parameter, and large values ($S_{33} > 0.7$) indicate limited conformational mobility. In combination with long-range couplings, the segmental order matrix serves as a qualitative measure of the mobility of the segment.

The segmental order tensor is related to the experimentally measured averaged NMR anisotropy parameters $\bar{\delta}$ according to

$$\bar{\delta} = {}^2\!/_3 \sum_{j=1}^{3}\sum_{k=1}^{3} S_{jk}\omega_{jk} \tag{2a}$$

where ω_{jk} are the elements of the NMR interaction tensor expressed in the same segment-fixed frame as S_{jk}. For a uniaxial interaction such as the dipolar coupling between two spins, this can be simplified in two ways. In the principal axis system (PAS) of the uniaxial NMR interaction, where $\omega_{33} = \delta$, $\omega_{22} = \omega_{11} = -{}^1\!/_2\delta$, and all off-diagonal elements are zero, we find

$$\bar{\delta} = S_{C-H} \times \delta \quad \text{with} \quad S_{C-H} = S_{33}^{\text{PAS}_{\text{NMR}}} = {}^1\!/_2\langle 3\cos^2\theta - 1\rangle \tag{2b}$$

where θ is the angle of the C–H bond with respect to the director. (The derivation is based on eq 1 and uses the relation $S_{11} + S_{22} = -S_{33}$.) This shows that the bond order parameter S_{C-H} is the scaling factor of the dipolar coupling constant. Alternatively, when evaluated in the PAS of the order tensor, eq 2a yields

$$\bar{\delta} = \delta\,{}^1\!/_2[S_{33}(3\cos^2\beta - 1) - (S_{22} - S_{11})\sin^2\beta\cos 2\alpha] \tag{2c}$$

where S_{ii} are the principal values of the order tensors (principal order parameters) and the angles β and α specify the polar coordinates of the internuclear vector in the PAS of the order tensor.

Generally, by measuring at least five averaged NMR anisotropies for each segment, the elements of the segmental order matrix can be determined by solving a system of at least five linear equations with five unknowns S_{jk}. The calculation can be considerably simplified by a suitable choice of the common coordinate system for the order matrix S_{jk} and the interaction tensors ω_{jk}, preferably according to segmental symmetries. The resulting order matrix is then diagonalized to obtain the principal values and the principal axis system. According to eq 2a, the order matrix contains complete information on the motionally averaged coupling constants of all segment-fixed dipolar interactions.

2. Orientation Distributions. The dynamic structure of lipid molecules and uniaxial liquid crystals can be analyzed in terms of the distributions of segmental orientations with respect to the director. But because of the macroscopic uniaxiality, the segmental orientation can be described completely by the distribution $P(\theta,\phi)$ of the director in a segment-fixed frame. To derive a functional form of the orientation distribution $P(\theta,\phi)$ that is related to the segmental order tensor **S**, one can consider the polar coordinates (θ,ϕ) in the PAS of the order tensor so that the principal values of the **S** tensor are

$$S_{33} = {}^1\!/_2\langle 3\cos^2\theta - 1\rangle = \sqrt{\frac{4\pi}{5}}\langle Y_{2,0}(\theta,\phi)\rangle =$$
$$\sqrt{\frac{4\pi}{5}}\int_0^{2\pi}d\phi\int_0^{\pi}d\theta\,\sin\theta\,P(\theta,\phi)\times Y_{2,0}(\theta,\phi) \tag{3}$$

and

$$S_{22} - S_{11} = {}^1\!/_2\langle 3\cos^2\gamma_2 - 1\rangle - {}^1\!/_2\langle 3\cos^2\gamma_1 - 1\rangle =$$
$${}^1\!/_2\langle\sin^2\theta\cos(2\phi)\rangle = \sqrt{\frac{2\pi}{15}}(\langle Y_{2,2}(\theta,\phi)\rangle + \langle Y_{2,-2}(\theta,\phi)\rangle) \tag{4}$$

It is well-known[16,17] that such averages $\langle Y_{L,M}(\theta,\phi)\rangle$ appear in the expansion of the distribution function $P(\theta,\phi)$ in terms of spherical harmonics $Y_{L,M}(\theta,\phi)$,

$$P(\theta,\phi) = \sum_{L=0}^{\infty}\sum_{M=-L}^{L}\langle Y_{L,M}(\theta,\phi)\rangle Y_{L,M}(\theta,\phi) \tag{5}$$

The first, or isotropic, term is $Y_{0,0} = 1/\sqrt{4\pi}$. All terms for odd L vanish due to the symmetry of the system under inversion of the director. Thus, only even-order terms remain. By truncating the series at $L = 2$, a low-resolution approximation $P^{(2)}(\theta,\phi)$ of $P(\theta,\phi)$ is obtained and can be expressed by the elements of the order matrix in the PAS:

$$P^{(2)}(\theta,\phi) = \langle Y_{0,0}\rangle Y_{0,0} + \langle Y_{2,0}(\theta)\rangle Y_{2,0}(\theta) +$$
$$\langle Y_{2,2}(\theta,\phi)\rangle Y_{2,2}(\theta,\phi) + \langle Y_{2,-2}(\theta,\phi)\rangle Y_{2,-2}(\theta,\phi) =$$
$$\sqrt{\frac{1}{4\pi}}Y_{0,0} + \sqrt{\frac{5}{4\pi}}S_{33}Y_{2,0} + \frac{1}{2}\sqrt{\frac{15}{2\pi}}(S_{22} - S_{11})(Y_{2,2}(\theta,\phi) + Y_{2,-2}(\theta,\phi)) \tag{6}$$

Here, the term ${}^1\!/_2 S_{12}(Y_{2,2}(\theta,\phi) - Y_{2,-2}(\theta,\phi))$ has vanished due to $S_{12} = 0$ in the PAS of the order tensor.

Figure 1a shows an example of an orientation distribution $P(\theta,\phi)$ with four maxima. It is represented by contour lines on the unit sphere. The corresponding $P^{(2)}(\theta,\phi)$ in Figure 1b yields only a low-resolution approximation to the distribution. In addition, for order tensors with large principal values, the $P^{(2)}(\theta,\phi)$ approximation contains aphysically negative regions. Better approximations that are positive everywhere can be made using model distributions described in the following.

3. Two-Parameter Distributions. Since the order matrix contains only five independent elements, a model distribution approximating $P(\theta,\phi)$ may contain no more than five parameters in order to be uniquely defined. Three angles parameterize the orientation of the distribution. This leaves only two adjustable shape parameters, which are directly related to S_{33} and $|S_{22} - S_{11}|$.

(a) Double Gaussian Distribution. A physically reasonable distribution with two shape parameters is the sum of two axially symmetric Gaussians of identical width σ, with their centers separated by an angle β, as shown in Figure 1c. This splitting β and width σ of the Gaussians are the two shape parameters of the distribution. If the orientational second moment of the individual Gaussian is S_M, then the principal order parameters of the total distribution fulfill the relations

$$S_{33} = S_M\,{}^1\!/_2(3\cos^2\beta - 1) \tag{7a}$$

and

$$|S_{22} - S_{11}| = S_M\,{}^3\!/_2|\sin^2\beta| \tag{7b}$$

The relation between the width σ of the Gaussian and the

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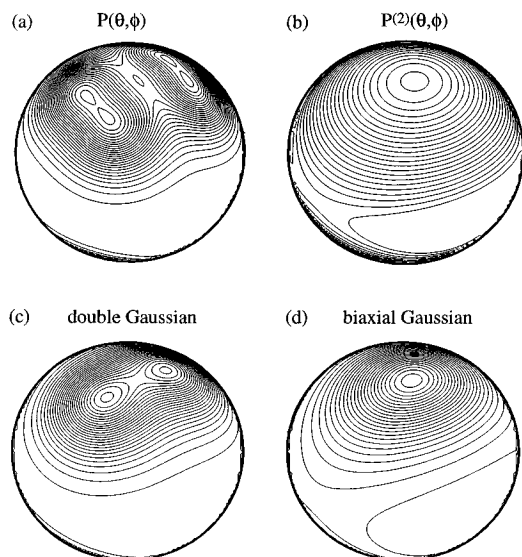*J. Phys. Chem., Vol. 100, No. 9, 1996* **3863**

Figure 1. Distributions of the director in a segment-fixed frame for order parameters $S_{33} = 0.4776$ and $S_{22} - S_{11} = 0.1206$. (a) The original distribution function $P(\theta,\phi)$ consists of four uniaxial Gaussians with widths of 15° and separated by 50° along the y axis and 35° along the x axis. (b) The $P^{(2)}(\theta,\phi)$ approximation obtained from the principal values of the order tensor. It exhibits negative intensities near $\theta = 90°$, which would be removed in higher-order approximations. (c and d) Five-parameter distributions that produce the same order tensor as the original distribution in (a). (c) The double Gaussian approximation. The two shape parameters are the separation of the two Gaussians ($\beta = 43°$) and the broadening ($\sigma = 17.5°$). (d) The single biaxial Gaussian approximation. The widths along the x and y directions are $\sigma_x = 25.0°$ and $\sigma_y = 19.6°$.

Figure 2. Relation between the principal order parameters (a) S_{33} and (b) $S_{22} - S_{11}$ and the two broadening parameters σ_x and σ_y of the biaxial Gaussian distribution. To determine σ_x and σ_y from the experimentally determined order parameters, one must find the identical pairs of σ_x and σ_y values in both plots that are consistent with the order parameters, as indicated by the dashed lines for the order parameters used in Figure 1.

corresponding S_M order parameter has been calculated and displayed by Hentschel et al.[17] It is also contained in Figure 2a, where S_M can be read from the S_{33} axis for $\sigma_x = \sigma_y = \sigma$. For a uniaxial order tensor, $|S_{22} - S_{11}| = 0$ and $\beta = 0$. This means the two Gaussians merge into one, making the distribution uniaxial as required.

This double Gaussian distribution is closely related to the model of exchange between two mirror-symmetric conformations, with S_M corresponding to the molecular order parameter. This will be discussed later.

(b) Single Biaxial Gaussian Distribution. A uniaxial Gaussian on a sphere, with its center at the north pole, is described by

$$P(\theta,\phi) = \exp\left(-\frac{\sin^2 \theta}{2\sigma^2}\right) = \exp\left(\frac{-(x^2 + y^2)}{2\sigma^2}\right) \qquad (8)$$

where $x = \sin \theta \cos \phi$ and $y = \sin \theta \sin \phi$ are the components of a vector of unit length. This formula can be generalized to produce a biaxial Gaussian,

$$P(\theta,\phi) = \exp\left(-\frac{x^2}{2\sigma_x^2} - \frac{y^2}{2\sigma_y^2}\right) \qquad (9)$$

The two broadenings σ_x and σ_y are the two shape parameters of this distribution. Figure 1d plots the biaxial Gaussian approximation to the original orientation distribution. Compared to the $P^{(2)}(\theta,\phi)$ function, it represents the features of the original orientation distribution with higher resolution. Figure 2 shows the relation between the shape parameters σ_x and σ_y of the biaxial Gaussian and the principal order parameters S_{33} and $S_{22} - S_{11}$, obtained by numerical integration. The limiting values of $S_{22} - S_{11}$ are found to be ± 0.75 and occur when ($\sigma_x \rightarrow \infty$, $\sigma_y \rightarrow 0$) and ($\sigma_x \rightarrow 0$, $\sigma_y \rightarrow \infty$). This can be understood as
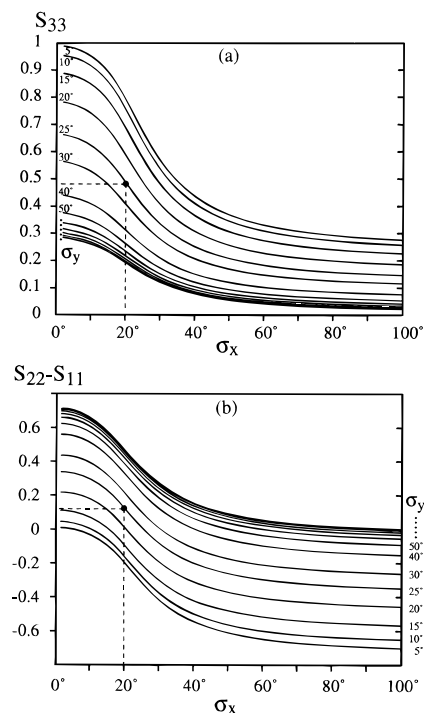
follows: on the unit sphere, the biaxial Gaussian with width parameters of $\sigma_x \rightarrow \infty$ and $\sigma_y \rightarrow 0$ has the shape of a narrow ring in the xz plane, perpendicular to the y axis (the S_{22} axis). With this extreme distribution, all directors are oriented at 90° from the y axis, and therefore, the principal value S_{22} must be $S_{22} = P_2(\cos 90°) = -0.5$. Since the distribution is uniaxial around the S_{22} axis, $S_{33} = S_{11} = -0.5S_{22} = 0.25$, and thus, $S_{22} - S_{11} = -0.75$. The case for $\sigma_y \rightarrow \infty$ and $\sigma_x \rightarrow 0$ is completely analogous and yields $S_{22} - S_{11} = 0.75$.

The width parameters of the two model distributions introduced here are related by $\min(\sigma_x,\sigma_y) \cong \sigma$ and $\max(\sigma_x,\sigma_y) \approx \sqrt{(\beta/2)^2 + \sigma^2}$. This is borne out by the parameters of the distributions shown in Figure 1.

Probability Limits from Order Parameters

In the preceding paragraphs, model distributions were parameterized based on the segmental order tensor. In the following, we will describe an approach for utilizing bond order parameters to obtain *model-independent* statements about bond orientation probabilities.

1. Model-Independent Probabilities from Negative Order Parameters. The bond order parameters S_{C-H}, obtained from the motionally-averaged C−H dipolar or ^{2}H quadrupolar coupling constants[1,10,12,18] according to eq 2b, contain information on the probability distribution of the angle between the C−H bond and the bilayer director. In particular, order parameters close to the limiting values of 1 and $-1/2$ contain much more information than the $L \leq 2$ approximation in the $Y_{L,M}$ expansion suggests. For instance, when $S_{C-H} = -0.5$, the θ dependence of the orientation distribution is fully known: it is a single narrow peak at $\theta = 90°$. This suggests that for S_{C-H} values close to -0.5, the orientation distribution will be high near $\theta = 90°$ and low near $\theta = 0°$. On the basis of this insight,
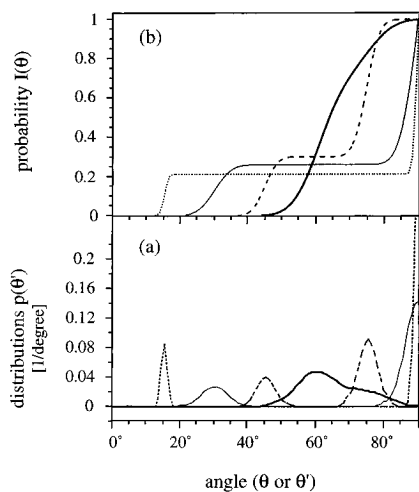
Figure 3. (a) Several possible distributions $p(\theta')$ that result in the bond order parameter $S_{C-H} = -0.2$, according to eq 11. (b) The corresponding integral orientational probabilities $I(\theta)$.

we ask the following question: given a negative order parameter, what is the upper limit to the probability of finding the bond oriented at small angles with respect to the director?

In the following, we will consider only the θ dependence of the orientation distribution. It is obtained from the two-dimensional distribution $P(\theta,\phi)$ by integrating over the angle ϕ:

$$p(\theta) = \frac{1}{2\pi}\int_{0°}^{2\pi}P(\theta,\phi)\sin\theta\,d\phi \qquad (10)$$

The bond order parameter S_{C-H} constrains the integral of $p(\theta)$ according to

$$S_{C-H} = \int_{0°}^{90°}p(\theta')P_2(\cos\theta')\,d\theta' \qquad (11)$$

Most S_{C-H} order parameters in the lipid molecule phosphocholine were found to be negative,[6,12] corresponding to angles θ_0, defined by $P_2(\cos\theta_0) = S_{C-H}$, which are larger than 54.7°.

Equation 11 suggests that the order parameter may yield information on the (integral) probability $I(\theta)$

$$I(\theta) = \int_{0°}^{\theta}p(\theta')\,d\theta' \qquad (12)$$

which is the probability of finding the bonds oriented at small angles from the director. Various examples of probability distributions $p(\theta')$ and the corresponding integrals $I(\theta)$, all corresponding to $S_{C-H} = -0.2$, are shown in Figure 3. As can be seen, it is impossible to give a model-independent limit for $p(\theta')$ on the basis of eq 11: $p(\theta')$ can in principle exhibit high and narrow spikes of small area that contribute little to the integral for S_{C-H}. In contrast, $I(\theta)$ is a steady function confined to values between 0 and 1. Still, there are many different functions $I(\theta)$ for a given S_{C-H}.

Our objective in the following is to find an upper-limit function, $I_{\lim}(\theta) \geq I(\theta)$, that provides as stringent a limit as possible to any $I(\theta)$ for a given negative order parameter. This is equivalent to searching for the extreme distribution $p_{\lim}(\theta')$ that (a) produces the maximum possible $I(\theta)$, according to eq 12, (b) yields the observed negative S_{C-H} value, according to eq 11, and (c) is normalized to unity.

Because S_{C-H} is negative, the distribution $p(\theta')$ must certainly contain a major peak at angles with negative $P_2(\cos\theta')$. On the other hand, it is possible that the bond orientation fluctuates
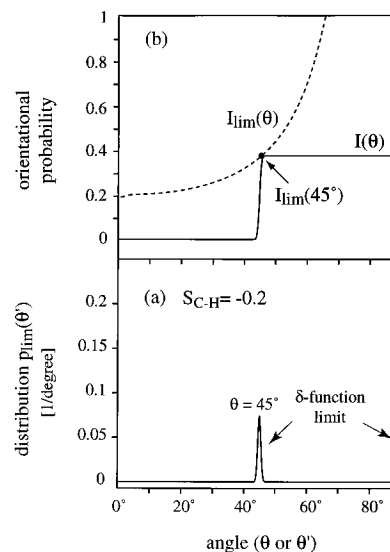

Figure 4. (a) Example of the limiting distribution function $p_{\lim}(\theta')$ for $S_{C-H} = -0.2$, for determining $I_{\lim}(45°)$. (b) The corresponding integral probability function $I(\theta)$ (solid line) provides one point on the upper-limit probability curve $I_{\lim}(\theta)$, at the θ value of the left peak in $p_{\lim}(\theta')$. This function $I_{\lim}(\theta)$ is indicated by the dashed curve.

to adopt smaller angles θ' with positive $P_2(\cos\theta')$. However, to produce the negative S_{C-H}, the probability for the smaller angles must have an upper limit. The probability $I(\theta)$ at small angles and large positive $P_2(\cos\theta')$ values will be largest if its contribution to S_{C-H} is compensated by a large $p(\theta')$ density in the region where $P_2(\cos\theta')$ is very negative. In the limiting case that we are looking for, $p_{\lim}(\theta')$ for $\theta' > \theta$ is a single peak at $\theta' = 90°$. On the other hand, in the region of $\theta' \leq \theta$, to maximize $I(\theta)$ while keeping $\int_{0°}^{\theta}p(\theta')P_2(\cos\theta')\,d\theta'$ constant, $p_{\lim}(\theta')$ has to be concentrated at the angle of the smallest allowed $P_2(\cos\theta')$. In other words, $p_{\lim}(\theta')$ must contain a narrow peak at θ. With the resulting extreme distribution

$$p_{\lim}(\theta') = I_{\lim}(\theta)\delta(\theta' - \theta) + (1 - I_{\lim}(\theta))\delta(\theta' - 90°) \quad (13)$$

shown in Figure 4a for $\theta = 45°$, eq 11 becomes

$$S_{C-H} = I_{\lim}(\theta)P_2(\cos\theta) + (1 - I_{\lim}(\theta))P_2(\cos 90°) \quad (14)$$

This yields the limit for the integral probability

$$I(\theta) \leq I_{\lim}(\theta) = \frac{S_{C-H} + 0.5}{P_2(\cos\theta) + 0.5} \qquad (15)$$

The model-independent upper-limit probability function, corresponding to an order parameter of $S_{C-H} = -0.2$, is shown as the dashed line in Figure 4b. The solid line in the figure is the integral $I(\theta)$ of the representative distribution $p_{\lim}(\theta')$ shown in Figure 4a. The plot indicates how the step of $I(\theta)$ determines the value of the $I_{\lim}(\theta)$ curve at 45°.

The model-independent upper-limit functions for a series of S_{C-H} values between 0.5 and −0.5 are calculated and plotted in Figure 5.

2. Probability Limits under the Assumption of Broadening. So far, the upper-limit function was found for a very aphysical distribution, consisting of two extremely narrow peaks. By assuming a reasonable broadening of the maxima of the distribution, an even more stringent upper limit to the integral probability can be obtained. A standard deviation σ of approximately 10°, corresponding to a molecular order parameter of 0.9,[17] appears as a safe minimum for the broadening in liquid crystals. In our calculations, we use Gaussian peaks
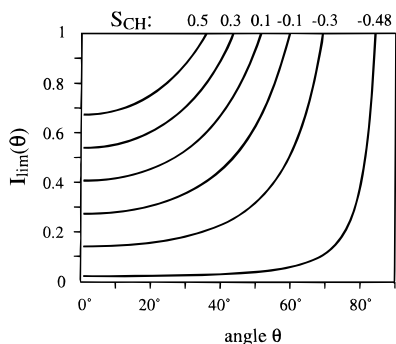
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Figure 5. $I_{lim}(\theta)$ parameterized by S_{C-H}. From this graph, the probability of finding the bond at small angles with respect to the director can be read off directly.

$$P(\theta,\phi) = 2\pi c \sum_j e^{-(\theta-\theta_j)^2/2\sigma_j^2} \Rightarrow p(\theta) = c \sin\theta \sum_j e^{-(\theta-\theta_j)^2/2\sigma_j^2} \tag{16}$$

with $p(\theta)$ normalized to unit integral by the constant c. However, the results do not depend strongly on the exact functional form of $p(\theta)$, as long as the standard deviations of the individual peaks are equal to σ.

It is difficult to find an analytical expression for the most stringent upper-limit probability function, $I_{lim}^{\sigma}(\theta)$, under the assumption of broadening. Instead, we performed a numerical calculation of $I_{lim}^{\sigma}(\theta)$. Again, the limiting distribution $p_{lim}^{10°}(\theta')$ consists of only two peaks (Figure 6a), one of which is centered at $\theta' = 90°$. The other peak has its maximum at an angle which is somewhat smaller than θ: a peak centered at θ will not produce the largest possible $I(\theta) = \int_{0°}^{\theta} p(\theta')\,d\theta'$, since only about 50% of that peak contributes to the integral. Thus, the integral will be larger if the peak is centered at an angle $\theta_c < \theta$, even though the amplitude of the peak will be smaller by approximately $P_2(\cos\theta_c)/P_2(\cos\theta)$. Note that although the limiting distribution contains only two peaks, the resulting $I_{lim}^{\sigma}(\theta)$ applies for any $p(\theta')$ function consisting of any number of Gaussian peaks with widths greater than σ. Shown in Figure 6b as a dashed line is the $I_{lim}^{\sigma}(\theta)$ curve with $\sigma = 10°$. It exhibits lower values than the $I_{lim}(\theta)$ curve at all angles, providing a more stringent upper limit to the probability of finding the bond below a certain angle with respect to the director. The quadratic θ dependence of $I_{lim}^{\sigma}(\theta)$ at $\theta \ll \sigma$ is due to the factor of $\sin\theta$ in the $p(\theta)$ function and to the fact that for $\theta \ll \sigma$ the integration covers only a part of the peak.

A set of $I_{lim}^{\sigma}(\theta)$ curves for different broadenings of the distribution, all with a bond order parameter of -0.22, is shown in Figure 7.

3. Limits for Positive Order Parameters. For positive order parameters, analogous considerations can be made to determine the integral probability between 90° and a minimum angle θ,

$$I_{90}(\theta) = \int_{\theta}^{90°} p(\theta')\,d\theta' \tag{17}$$

In this case, $p(0°)P_2(\cos 0°)$ compensates for the contributions of negative P_2 values to S_{C-H} in eq 11. In analogy to eq 14, the upper limit to the probability of finding the C−H bonds above a certain angle θ with respect to the director is determined by

$$S_{P-C} = I_{90,lim}(\theta)P_2(\cos\theta) + (1 - I_{90,lim}(\theta))P_2(\cos 0°) \tag{18}$$

where the index "P−C" is used because large positive order parameters are observed for $^{31}P-^{13}C$ dipolar couplings in
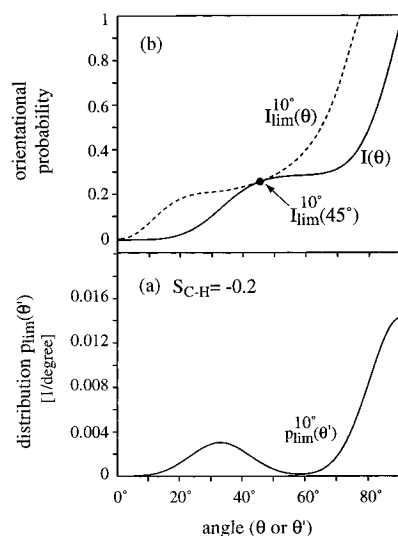

Figure 6. (a) Example of the limiting distribution function $p_{lim}^{10°}(\theta')$ with 10° minimal broadening and $S_{C-H} = -0.2$. (b) The corresponding integral probability $I(\theta)$ (solid line) and the upper-limit probability curve $I_{lim}^{10°}(\theta)$ (dashed line).

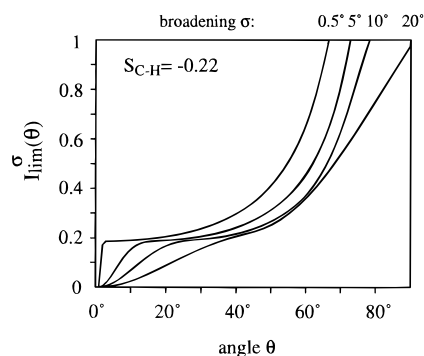**Figure 7.** $I_{lim}(\theta)$ parameterized by σ, the minimal Gaussian broadening of the distribution function. The order parameter $S_{C-H} = -0.22$.

lipids.[19] This yields

$$I_{90}(\theta) \le I_{90,lim}(\theta) = \frac{1 - S_{P-C}}{1 - P_2(\cos\theta)} \tag{19}$$

The integral-limit probability curve for $S_{P-C} = +0.4$, as found for the vector connecting the ^{31}P and the glycerol C3 nuclei in phosphocholine lipids, is shown in Figure 8a (solid line). Although not as stringent as that for $S_{C-H} = -0.2$, it still shows that the angle between the P−C3 vector and the director is preferentially smaller than 63°.

When a minimal broadening of the distribution is assumed, the integral probability curve $I_{90,lim}^{\sigma}(\theta)$ is shifted to smaller angles, as shown in Figure 8a by the dashed line. Thus, the upper limit of the integral probability of finding the bond orientation between an angle θ and 90° is more stringent. Examples of the probability density functions $p_{90,lim}^{\sigma}(\theta')$ determining the limiting values at $\theta = 45°$ are shown in Figure 8b. From their integrals, maximal probabilities of the bond being oriented at angles larger than 45° were found to be 76% for the δ function limit and only 50% in the case of 10° broadening.

4. Model-Independent Probabilities from Chemical Shift Anisotropies. It may be interesting to note that it is possible to extend the model-independent treatment to motionally averaged chemical shift anisotropies. This could be of interest, for instance, for the ^{31}P chemical shift anisotropy of the
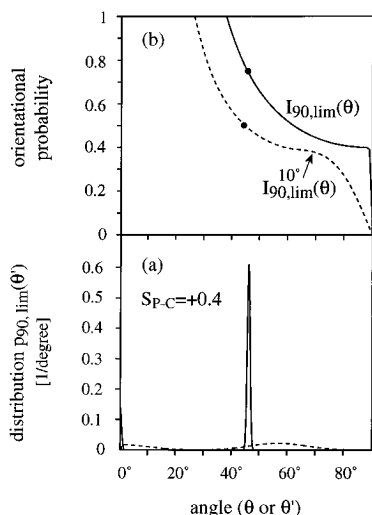
Figure 8. (a) Upper-limit probability functions $I_{90,\lim}(\theta)$ and (b) the corresponding limiting distribution functions $p_{90,\lim}(\theta')$ for a positive order parameter $S_{P-C} = +0.4$. The integrals of the distribution functions yield the points at $\theta = 45°$ on the $I_{90,\lim}(\theta)$ curves. Solid lines: the model-independent case. Dashed lines: with 10° minimal Gaussian broadening.

phosphate in the headgroup of phospholipids, whose rigid-limit chemical shift tensor orientation is well-established.[20] Since the chemical shift tensor is generally not axially symmetric ($\eta \neq 0$), we start from the expression of eq 2a in the chemical shift PAS:

$$\bar{\delta} = \delta \tfrac{1}{2} \langle 3 \cos^2 \theta - 1 - \eta \sin^2 \theta \cos 2\phi \rangle \quad (20)$$

where $\delta = \sigma_{33}$ is the chemical shift principal value of the largest magnitude (z axis) and $\eta = |\sigma_{11} - \sigma_{22}|/\sigma_{33}$. The angles θ and ϕ are the polar coordinates of the director in the chemical shift PAS. We consider the case where $\bar{\delta}$ and δ have opposite signs. The extreme distribution with the largest $I(\theta)$ for the given $\bar{\delta}/\delta$ consists again of two sharp peaks, one at $\theta' = \theta$ (in the plane of $\phi = 0°$, as it turns out) and the other at the chemical shift value furthest away from $\delta = \sigma_{33}$, which is $\sigma_{11} = -(\delta/2)(1 + \eta)$. Then, the equivalent of eq 14 is found:

$$\bar{\delta}/\delta = I_{\lim}(\theta) \tfrac{1}{2}(3 \cos^2 \theta - 1 - \eta \sin^2 \theta) +$$
$$(1 - I_{\lim}(\theta))\left(-\frac{1}{2}(1 + \eta)\right) \quad (21)$$

This yields the limit for the integral probablity

$$I(\theta) \leq I_{\lim}(\theta) = \frac{\bar{\delta}/\delta + \tfrac{1}{2}(1 + \eta)}{\tfrac{1}{2}(3 \cos^2 \theta + \eta - \eta \sin^2 \theta)} \quad (22)$$

which for $\eta = 0$ reduces to eq 15, as required.

Discussion

The analysis of order tensors in terms of the five-parameter distribution functions is valuable for the elucidation of molecular structure and dynamics when segmental tensors are known for many sites in the molecule. For determining the torsional angles between two segments, the order tensors of both sites must be known. It is interesting to note that specific constraints are provided by the bonding of the two sites, since the order tensor of either site must yield the same order parameter along the direction of the connecting bond. In addition, the relative orientation of these two tensors is partly fixed by this common axis. Only the torsional angle around this axis needs to be

determined. This is possible in favorable cases of limited conformational dynamics, which will be discussed in the following.

If the molecule is rigid, all segmental order tensors are uniaxial and have the same unique principal value, the molecular order parameter S_M. Of course, due to different segmental orientations in the molecule, the C–H and other bond order parameters can still take the full range of values between S_M and $-S_M/2$. In a rigid molecule, the torsional angle between two bonded segments can be determined by superimposing both the S_{33} axes (i.e., the maxima of the $P(\theta,\phi)$ distributions) and the connecting bond directions of the two segments. Only when the C–C and S_{33} directions coincide will this procedure fail.

Internal dynamics of the molecule is proved by highly nonuniaxial order tensors. The simplest model of internal dynamics of the molecule is exchange between two mirror-symmetric conformations. Such an exchange was first proposed by Seelig et al.[7] for the headgroup region of phosphocholine to explain ^{2}H NMR data. It corresponds to an inversion of all torsional angles in that region and is therefore consistent with the symmetry of the intramolecular potentials. To verify the extent to which such a two-conformation model is valid, the parameters of the double Gaussian distribution introduced above are of great use. If the two-site model is valid, the widths of the Gaussians must be the same for all segments involved. Then, by aligning both the maxima of the Gaussians and the C–C bond directions for the adjacent segments, the torsional angle is constrained to four values, namely, pairs of values with opposite signs. One of these pairs is the correct solution. Variation of the widths of the Gaussian peaks in the model distribution proves the presence of more complex internal dynamics. For instance, a systematic increase of the Gaussian width parameter toward the chain end may suggest a mobility gradient.

The value of the upper-limit probability analysis introduced above can be illustrated by the example of the two C–H bonds of the glycerol *sn*-3 site. They were both found to have bond order parameters of -0.22. If only one bond orientation relative to the director was present, this bond order parameter would correspond to an angle of $\theta_0 = 64°$, since $P_2 (\cos 64°) = -0.22$. Obviously, this assumption of a single orientation is unrealistic. However, by using eq 14, we can state, independent of any mode, that the two C–H bonds are found to orient preferentially (i.e., with a probability of more than 50%) at angles larger than 50° from the director. The probability for angles smaller than 30° is less than 25%. If we include the physically reasonable assumption that any maximum in the distribution has a standard deviation of at least 10° (but still not making any specific asumnptions on the conformations taken by the molecule), then the probability of the bond being oriented at angles smaller than 51° from the director is less than 25%, according to the curve in Figure 7.

Above, we also showed how the model-independent treatment can be extended to chemical shift anisotropies. As an example, we consider the ^{31}P chemical shift anisotropy, with $\bar{\delta}/\delta = -0.25$ and $\eta = 0.56$. The model-independent calculation shows that the vector connecting the non-esterified oxygens of the phosphate unit, which is the σ_{33} axis of the ^{31}P chemical shift tensor, is preferentially oriented between 40° and 140° from the director.

Summary

We have presented two complementary approaches to obtain structural information on liquid-crystalline lipids from segmental order parameters measured by NMR. The analysis in terms of the orientational probability limits yields model-independent

information from bond order parameters with large negative (< -0.15) or positive (>0.4) values. This gives us some insight into the bond orientation. For instance, this model-independent analysis shows that, with bond order parameters of -0.22, the C$-$H bonds of the glycerol *sn*-3 CH$_2$ group in phosphatidylcholine are oriented preferentially at angles between 52° and 128° from the director. The probability of these bonds being oriented at angles smaller than 30° or larger than 150° is less than 25%. However, this approach offers little information on the torsional angles in the molecule. Such information can be obtained from a full order tensor analysis, provided the internal dynamics of the molecule is not too complex. The presence of various types of dynamics can be inferred by comparing the order tensors of several segments. For obtaining information on the torsional angle that characterizes the relative orientation of two segments, five motionally averaged coupling constants of segment-fixed NMR interactions need to be measured for each segment. Although a large number of homo- and heteronuclear couplings have been determined for the headgroup region of phosphocholine lipids,[10,12,19] still more are required for the full order tensor analysis. The ideas presented here are also applicable to uniaxial liquid-crystalline systems other than lipids.

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