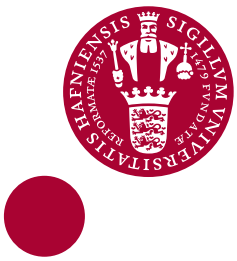

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Relationships between lipid membrane area, hydrophobic thickness, and acyl-chain orientational order

The effects of cholesterol

John Hjort Ipsen,* Ole G. Mouritsen,* and Myer Bloom‡

*Department of Structural Properties of Materials, The Technical University of Denmark, DK-2800 Lyngby, Denmark; and
‡Department of Physics, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

ABSTRACT A microscopic interaction model for a fully hydrated lipid bilayer membrane containing cholesterol is used to calculate, as a function of temperature and composition, the membrane area, the membrane hydrophobic thickness, and the average acyl-chain orientational order parameter, S. The order parameter, S, is related to the first moment, M_1, of the quadrupolar magnetic resonance spectrum which can be measured for lipids with perdeuterated chains. On the basis of these model calculations as well as recent experimental measurements of M_1 using magnetic resonance and of membrane area using micromechanical measurements, a discussion of the possible relationships between membrane area, hydrophobic thickness, and moments of nuclear magnetic resonance spectra is presented. It is pointed out that S under certain circumstances may be useful for estimating the hydrophobic membrane thickness. This is particularly advantageous for multicomponent membranes where structural data are difficult to obtain by using diffraction techniques. The usefulness of the suggested relationships is demonstrated for cholesterol-containing bilayers.

INTRODUCTION

Whereas a substantial amount of experimental information is available on the thermodynamic properties of lipid bilayers (Silvius, 1982) and biological membranes (Keough and Davis, 1984), the existing data for the mechanical and geometrical properties of membranes is more limited (Evans and Needham, 1987). There are reasons for this. Not only is it usually easier to conduct thermodynamic measurements (e.g., by differential scanning calorimetry) on membranes, but it is also simpler to provide a physical interpretation of the results obtained by such experiments. In contrast, experimental measurements of the mechanical properties are considerably more difficult (Evans and Skalak, 1980; Lis et al., 1982 *a* and *b*) and only within recent years have some reliable data on lateral area compressibility been obtained for a few model membrane systems, mainly by use of micropipet pressurization of individual single-walled lipid vesicles (Kwok and Evans, 1981; Evans and Kwok, 1982; Needham et al., 1988). As for the geometrical properties of lipid bilayers and membranes, specifically the bilayer hydrophobic thickness and the average molecular cross-sectional area, the available data are also surprisingly scarce (Inoko and Mitsui, 1978; Zaccai et al., 1979; Dilger et al., 1982; Lewis and Engelman, 1983; Sadler et al., 1984; Needham et al., 1988). One reason for this is that a direct determination of the bilayer thickness by diffraction methods requires oriented membrane samples with sufficiently high long-range order to provide several orders of Bragg reflection. Moreover, estimates of the size of the polar-head-group region and the position of the hydrophilic/hydrophobic boundary are uncertain and ambiguous (Scherer, 1989) which makes the determination of the hydrophobic thickness from diffraction data uncertain. Obviously there is at present a severe lack of experimental information on some very basic physical properties of membranes.

This lack of experimental data on membrane mechanical and geometrical properties is unfortunate and should be remedied because there is accumulating evidence that such properties modulate lipid-protein interactions in membranes (Bloom and Smith, 1985) and moreover regulate a variety of membrane functions (Sackmann, 1984). In a recent theoretical analysis (Mouritsen and Bloom, 1984) we have pointed out that the degree of matching between lipid-bilayer and integral-protein hydrophobic thicknesses is an important aspect of lipid-protein interactions. A great variety of experiments on systems ranging from ion-channels, ATPases, and other enzymes (for a recent review, see e.g., Mouritsen, 1986) moreover indicate that this degree of matching regulates the activity of many membrane-bound molecules. Adding to this the striking piece of information that special molecules, of which cholesterol is the most prominent example, may regulate membrane function indirectly by changing the membrane hydrophobic thickness (Stephens and Shinitzky, 1977; Bloom and Mouritsen, 1988), it becomes clear that it is important to deal seriously with the lack of data for, e.g. membrane thicknesses.

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In this paper we shall explore an indirect method of experimentally determining the hydrophobic bilayer thickness, d, in the liquid-crystalline phase, L_α, from measurements of deuterium (^2H) nuclear magnetic resonance (NMR) orientational order parameters (S_{CD}) of CH-bonds on the acyl chains of phospholipid molecules. Denoting the angle between a CH-bond on the ith carbon on the acyl chain and the bilayer normal by Θ_i, its order parameter is defined by the average overall chain conformations

$$S_{CD}^{(i)} = \tfrac{1}{2}\overline{(3\cos^2\Theta_i - 1)}. \quad (1)$$

As we shall discuss later, $S_{CD}^{(i)}$ is related via the geometry of acyl chains to the orientational order parameter of the ith C—C bond. Since the vector joining the two ends of the acyl chain is obviously the sum of the C—C bond vectors, one would certainly anticipate a correlation between d and the average orientational order parameter, $\langle S_{CD}\rangle$, for an acyl chain having N carbon atoms, i.e., for $COO(CH_2)_{N-2}CH_3$,

$$\langle S_{CD}\rangle = \frac{1}{N-1}\sum_{i=2}^{N} S_{CD}^{(i)}. \quad (2)$$

In fact, a linear relationship between d and $\langle S_{CD}\rangle$ has been derived by J. Seelig and collaborators under the assumption that orientational order arises from conformational and rotational motions of axial symmetry about the bilayer normal (Seelig and Seelig, 1974, 1980; Schindler and Seelig, 1975). In practice, the measurement of ^2H-NMR quadrupolar splittings provides values of $|S_{CD}^{(i)}|$ rather than $S_{CD}^{(i)}$. For this reason, we express the linear relationship in the three papers cited above in the form

$$d = d_1(\alpha\langle|S_{CD}|\rangle + \beta), \quad (3)$$

where α and β are constant coefficients, d_1 is the maximum value of d, and

$$\langle|S_{CD}|\rangle = \frac{1}{N-1}\sum_{i=2}^{N}|S_{CD}^{(i)}|. \quad (4)$$

Obviously, Eq. 3 is only a consequence of Seelig's calculation in cases where the $S_{CD}^{(i)}$ all have the same sign. This is usually the case for saturated acyl chains. A linear relationship between d and $\langle|S_{CD}|\rangle$ does not necessarily hold in the general case since Eq. 3 was derived for a lattice model (Seelig and Seelig, 1974, 1980; Schindler and Seelig, 1975) which is certainly a simplified picture of the L_α phase. A related but differently based relationship between d and ^2H-NMR quadrupolar splittings has been proposed by De Young and Dill (1988). Ultimately, the degree to which Eq. 3 represents a useful approximation can only be established via x-ray and neutron diffraction studies. Thus far, comparative diffraction and $\langle|S_{CD}|\rangle$ data are only available for a single system, dipalmitoyl phosphatidylcholine (DPPC) (Seelig and Seelig, 1974, 1980; Zaccai et al. 1979) for which Eq. 3 seems to be adequate (Bloom and Mouritsen, 1988).

Here, we investigate the consequences of Eq. 3 for fully hydrated mixtures of DPPC and cholesterol on which careful ^2H-NMR and other experimental studies (Vist, 1984; Davis, 1988; Needham et al., 1988; Vist, M. R., and J. H. Davis, manuscript submitted for publication; see also references to experimental work in the theoretical paper by Ipsen et al., 1987) have provided a partial phase diagram as well as the variation of various thermomechanic parameters with temperature and composition. The DPPC-cholesterol phase diagram and the physical properties of the different phases have been explained in terms of a microscopic interaction model (Ipsen et al., 1987; Cruzeiro-Hansson et al., 1989). We present in this paper some theoretical results based on an extension of this model which predicts values of d as a function of temperature and cholesterol concentration and also values of the α and β parameters in Eq. 3. It is hoped that our discussion of the relationship between the predictions and the experimental data on $\langle|S_{CD}|\rangle$ will stimulate diffraction studies on this and related bilayer systems.

MODEL AND METHODS OF CALCULATION

The microscopic interaction model for lipid-cholesterol membranes used in this work is an extended version of the model proposed by Ipsen et al. (1987) to describe the phase equilibria in the phosphatidylcholine-cholesterol system. The model of Ipsen et al. (1987) is based on an earlier model for the main phase transition in pure lipid monolayers (Mouritsen and Zuckermann, 1987) and bilayers (Zuckermann and Mouritsen, 1987) which in turn is a combination of two different lattice models, (*a*) the two-state model of Doniach (1978) and (*b*) a site-diluted high-Q-state Potts model (Mouritsen and Zuckermann, 1987). These two models were employed to account for two types of ordering processes which can take place in lipid membranes, (*a*) conformational ordering of the acyl chains via rotational isomerism and van der Waals interactions, and (*b*) two-dimensional crystallization of the lipid molecules in the plane of the membrane via translational degrees of freedom. The two models may be considered as respectively describing the internal and the lateral degrees of freedom of the membrane molecules. The Doniach model ascribes two different conformational states to the acyl chains with different internal entropies, and it is the entropy difference between these two states which drives the first-order chain-melting transition. The high-Q-state Potts model describes the fact that the acyl chains can crystallize into a large number, Q, of different orientations labeled by the Q Potts states. The Potts interaction then accounts for the energy in the packing defects and the domain boundaries which arise when crystallites of different orientations meet. The variables of the Potts model therefore characterize in a very approximate manner the ordering in the translational degrees of freedom.

To this model of the phase transition in pure lipid membranes a set of lipid-cholesterol interactions was added (Ipsen et al., 1987) which reflect the following facts: (*a*) cholesterol prefers to be dissolved in fluid (noncrystalline) phases, and (*b*) cholesterol prefers to have conforma-

tionally ordered acyl chains next to it. (*a*) was modeled by decoupling the Potts interaction between nearest-neighbor sites whenever a cholesterol molecule is involved. (*b*) was modeled by assigning to cholesterol a hydrophobic shape which implies that the van der Waals coupling to a lipid acyl chain is stronger the more the chain is conformationally ordered. As was shown by Ipsen et al. (1987), such a model includes the necessary physics to explain the experimentally observed phase equilibria in the DPPC-cholesterol system. In particular it accounts for the very modest freezing point depression up to ~8 mol% cholesterol and the massive phase separation which sets in for higher concentrations. Furthermore, the model describes the particular homogeneous cholesterol-rich phase above ~20 mol% as a fluid phase with a high degree of chain-conformational order.

In the present work we have used an extension of the model described above by adopting the 10-state model due to Pink et al. (1980; 1981) instead of the Doniach model. This extended model of lipid-cholesterol interactions was recently used to describe the thermal anomalies in the specific heat of lipid bilayers containing cholesterol (Ipsen et al., 1989). The ten-state model takes accurate account of a larger number of chain states and their conformational statistics. This more refined description of the phase transition is required for calculating the acyl-chain orientational order parameters. The ten conformational states were selected by Pink et al. (1980) from requirements of low conformational energy and good packing properties. The basis assumption is that the first three-chain segments are fixed along the bilayer normal and that the remaining segments can only have C—C-bond angles of 120°. Each of the ten chain-conformational states of the model is characterized by a conformational energy, a conformational entropy, and a cross-sectional area, A_n, $n = 1, 2, \ldots, 10$. It is furthermore assumed that the hydrophobic volume occupied by each chain is a constant (Marčelja, 1974) and hence

$$A_n = \frac{d_1 A_1}{d_n}, \tag{5}$$

where d_n is the hydrophobic chain length and A_1 is the cross-sectional area of the all-*trans* state, i.e. $A_1 = 40.8$ Å^2 (Tardieu et al., 1973). The molecular cross-sectional area of cholesterol is taken to be $A_C = 32$ Å^2 as estimated from x-ray work (Engelman and Rothman, 1972).

In the Pink model, the van der Waals interaction between two molecules is proportional to a product of shape factors, $V_n V_m$. In the case of cholesterol the shape factor is simply a constant, reflecting the fact that cholesterol is a hydrophobically smooth molecule with no appreciable internal flexibility. In the case of a lipid molecule, the shape factor is the average acyl-chain order parameter (segmental order parameter) (Wulf, 1977)

$$V_n = S_n = \frac{1}{2(N-1)} \sum_{i=2}^{N} (3 \cos^2 \vartheta_{ni} - 1), \tag{6}$$

where the summation extends over all segments of the chain and ϑ_{ni} is the angle between the bilayer normal and the normal to the plane spanned by the ith CH_2-group of the nth selected conformational state of the acyl chain. For the completely ordered all-*trans* state, $S_1 = 1$. The average acyl-chain order parameter can therefore readily be derived from the model using

$$S = \sum_n p_n(T, x) S_n, \tag{7}$$

where $p_n(T, x)$ is the temperature- and composition-dependent thermodynamic probability of the nth chain state. Due to the model assumption that only chain conformations are allowed which have C—C bond angles of 120°, it follows from geometry that

$$S_n = a d_1^{-1} d_n + b, \tag{8}$$

where $a = 1.8$ and $b = -0.8$ are geometrical factors. This implies that the hydrophobic thickness within the present model is linearly related to the average acyl-chain order parameter

$$d = \sum_n p_n(T, x) d_n = (S - b) a^{-1} d_1. \tag{9}$$

The hydrophobic length of the chain in the all-*trans* state is taken to be $d_1 = 19.7$ Å for DPPC (Marčelja, 1974).

The thermodynamic properties of the microscopic interaction model described above are calculated within the mean-field approximation (Ipsen et al., 1987). From the free energy the phase diagram is readily derived. The chain-state probability function, $p_n(T, x)$, is used to calculate the average cross-sectional area per molecule, $A = \Sigma_n p_n(T, x) A_n$, the hydrophobic thickness, d in Eq. 9, and the average chain orientational order parameter, S in Eq. 7.

Before presenting the results of the model calculations we wish to emphasize that the goal of the present modeling is not to search for a set of optimized model parameters for the accurate quantitative description of the lipid-cholesterol system. At present, the parameters of the model (Ipsen et al., 1987) are known with too little confidence to make this goal worth pursuing. The main reason for this is the lack of very accurate experimental information on the phase diagram to which the model parameters should be fitted. Furthermore, the mean-field approximation is too imprecise to yield quantitatively reliable results in the transition region even for a model with the optimized parameter values. Rather, it is our intention to provide a theoretical prediction of the overall qualitative variation of the thermomechanic properties of the mixture across the phase diagram. The set of model parameters used for the lipid molecules is pertinent to acyl chains with 16 monomers as far as the conformational statistics and the van der Waal interactions are concerned. In this respect they correspond to the properties of DPPC neglecting special effects such as chain tilting and the occurrence of rippled phases.

MODEL RESULTS

Membrane area, hydrophobic thickness, and orientational order parameters

In Fig. 1 the phase diagram is shown as derived from the microscopic model. This diagram is very similar to that obtained from a two-state model (Ipsen et al., 1987). The diagram contains three homogeneous phases, the solid-ordered (so) phase, the liquid-disordered (ld), and the liquid-ordered (lo) phase. The two phase labels refer respectively to the lateral nature of the two-dimensional membrane phase and the average internal conformational state of the acyl chains. For the pure system, so and ld are identical to the so-called gel and fluid (liquid-crystalline) phases. The physical effect of cholesterol is to disrupt the lipid crystalline solid and to induce conformational order in the fluid. These two competing effects balance each other up to ~8 mol% cholesterol where the cholesterol-induced orientational order in the fluid phase has reached

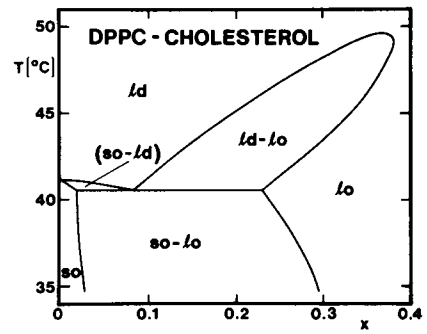

FIGURE 1 Theoretical phase diagram for DPPC bilayers containing cholesterol. The various phases are labeled by so (solid-ordered), ld (liquid-disordered), and lo (liquid-ordered).

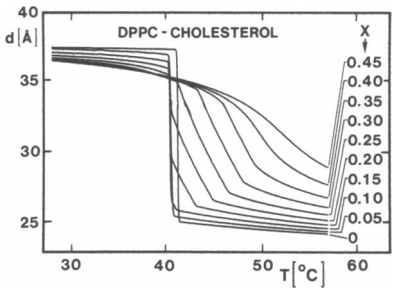

FIGURE 3 Hydrophobic thickness, d, of a DPPC bilayer containing cholesterol. Theoretical results for d are shown as a function of temperature for different cholesterol concentrations, x.

a level where cholesterol prefers this new phase, lo. Massive phase separation then sets in both at low and high temperatures on both sides of the three-phase line. There is a eutectic point at $x_{eu} \simeq 0.08$. The phase diagram has an upper critical point beyond which the ld and lo phases become equivalent.

The average cross-sectional area per molecule of the mixture, A, is shown in Fig. 2. It is seen that the transition is broadened in the presence of cholesterol for $x < x_{eu}$, and shifted towards slightly lower temperatures. For $x > x_{eu}$, A has two distinct features: (a) a sharp change at the three-phase line; this change decreases towards zero as the terminus of the three-phase line is approached at $x \simeq 0.23$. (b) a flattened portion of A corresponding to crossing the (ld-lo) coexistence region. The data of Fig. 2 clearly demonstrate the strong condensing effect of cholesterol above the pure bilayer main transition. At low temperatures, the effect of cholesterol on A is much less pronounced and there is a rather complicated behavior in a temperature region immediately below the main transition. However, at low concentrations the expansion effect of cholesterol is clearly seen. This complicated variation is caused by the mere dilution effect of cholesterol whose molecular cross-sectional area is closer to that of the lipid-ordered chains than that of disordered chains.

Cholesterol's expansion and condensation effects are concomitantly reflected in the data for the hydrophobic bilayer thickness (or average acyl-chain length) shown in Fig. 3. The bilayer is thinned by cholesterol below the main transition and thickened above the main transition. The data plotted for d in the two-phase region is the average hydrophobic membrane thickness. In principle, d is a double-valued function within the two-phase region reflecting the fact that the ld and lo phases have different thicknesses below the critical point (for a similar effect in lipid-lipid mixtures, see Ipsen and Mouritsen [1988]).

Finally, these same effects are seen to be reflected in the average acyl-chain order parameter, S, in Fig. 4. Cholesterol disorders the lipid chains below the main transition and orders the chains above the main transition. It should be pointed out that these effects on the acyl-chain orientational order as they come out of our model calculations are a highly nontrivial result since the simple lipid-cholesterol interactions of the model are nonspecific and only operate at the level of hydrophobic contact (van der Waals) interactions. In particular, it

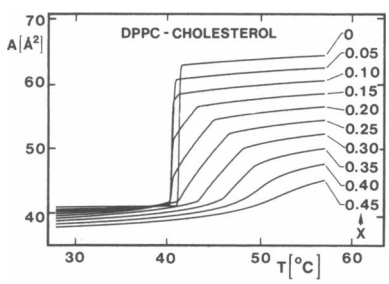

FIGURE 2 Average cross-sectional area, A, per molecule of a DPPC bilayer containing cholesterol. Theoretical results for A are plotted as a function of temperature for different cholesterol concentrations, x.

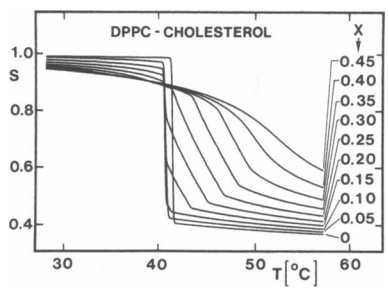

FIGURE 4 Average acyl-chain order parameter, S in Eq. 5 for a DPPC bilayer containing cholesterol. Theoretical results for S are plotted as a function of temperature for different cholesterol concentration, x.

should be noted that the model is not based on any kind of lipid-cholesterol complexing (Ipsen et al., 1989).

RELATIONSHIP BETWEEN HYDROPHOBIC THICKNESS AND NMR ORDER PARAMETERS

In the Introduction we have reviewed the linear relationship between d and $\langle|S_{CD}|\rangle$ as proposed by Seelig and Seelig (1974, 1980) and Schindler and Seelig (1975). Here, we comment on the experimental determination of acyl-chain orientational order and the range of validity of the postulated linear relation.

An important assumption in the derivation of Eq. 3 is that orientational order arises from conformational and rotational motions which have axial symmetry about the bilayer normal. For this reason, Eq. 3 may be used directly to determine the hydrophobic thickness of the L_α-phase but not that of the $L_{\beta'}$- and $P_{\beta'}$-phases in which the acyl chains are tilted with respect to the bilayer normals.

The maximum possible value of the hydrophobic thickness is associated with molecules in the all-*trans* conformation of acyl chains oriented parallel to the bilayer normal. Then, rotation about the bilayer normal combined with the acyl-chain geometry dictates that $S^{(i)}_{CD} = -\frac{1}{2}$ for all i. Many workers in this field identify the quantity $2|S^{(i)}_{CD}|$ with a local 'molecular order parameter', $S^{(i)}_{mol}$; because it is anticipated that for $d < d_1$, in the L_α-phase, all $S^{(i)}_{CD}$ will have values in the range $-\frac{1}{2} \leq S^{(i)}_{CD} \leq 0$ (Seelig and Seelig, 1980). Therefore, the coefficients α and β in Eq. 3 should both be positive and satisfy the condition

$$0.5\alpha + \beta = 1. \tag{10}$$

Values of α and β used thus far to compare $\langle|S_{CD}|\rangle$ with x-ray and neutron diffraction determinations of d are $\alpha = \frac{10}{9}$ and $\beta = \frac{4}{9}$ (Seelig and Seelig, 1974, 1980; Zaccai et al., 1979) or $\alpha = 1$ and $\beta = \frac{1}{2}$ (Schindler and Seelig, 1975; Bloom and Mouritsen, 1988). Comparing $\langle|S_{CD}|\rangle$ with the definition of S in Eqs. 7 and 8 we are led to identify S with $S^{(i)}_{mol}$ and to put $S = S^{(i)}_{mol} = \langle|S_{CD}|\rangle$. Thus, also the microscopic model gives $\alpha = \frac{10}{9}$ and $\beta = \frac{4}{9}$. Both sets of coefficients, α and β, are in agreement with the available diffraction data. More appropriately, one can say that the overlap of good quality diffraction data and ^{2}H-NMR data is too sparse to provide a check of the general validity of Eq. 3, let alone to distinguish between the two sets of coefficients.

A simple method of determining $\langle|S_{CD}|\rangle$ is via its relationship with the 'first moment', M_1, of the ^{2}H-NMR 'half-spectrum' of phospholipid molecules in which all the acyl-chain protons have been replaced by deuterons (Davis, 1979, 1983; Davis et al., 1980)

$$\langle|S_{CD}|\rangle = \frac{3\sqrt{3}}{4\pi}\frac{M_1}{\delta\nu_{max}}, \tag{11}$$

where $2\delta\nu_{max}$ is the maximum value of the quadrupolar splitting for a deuteron on a CH-bond. The use of a quadrupolar coupling constant of 167 kHz (Davis, 1979) yields $\delta\nu_{max} = 125$ kHz.

Davis and Vist (Vist, 1984; Davis, 1988; Vist, M. R. and J. H. Davis, 1990) have measured M_1 for mixtures of d_{62}-DPPC and cholesterol from which $\langle|S_{CD}|\rangle$ and, hence, the bilayer thickness may be estimated using Eqs. 3 and 11. The values of $2\langle|S_{CD}|\rangle$ as a function of temperature are shown in Fig. 5 for DPPC-cholesterol mixtures for several cholesterol concentrations in the range studied by Davis and Vist. Note that the transition temperature of d_{62}-DPPC bilayers is close to $T = 37$°C compared with $T = 41$°C for protiated DPPC bilayers. For the pure DPPC sample, $x = 0$, the variation of M_1 with temperature just below 37°C is characteristic of the $P_{\beta'}$ phase while that below ~25°C is associated with the $L_{\beta'}$ phase. The shapes of the ^{2}H NMR spectra below 37°C reflect the nonaxially symmetric nature of the molecular motions in these phases (Davis, 1979) so that Eq. 3 is not applicable to DPPC in this region. Indeed, the values of $\langle|S_{CD}|\rangle$ exceed 0.5 for $T < 0$°C (Davis, 1979) so that Eq. 3 would predict the nonphysical result that $d > d_1$ in that range. Since the ^{2}H NMR spectra for the $x = 0.225$ sample are characteristic of axially symmetric molecular motions for $T > 20$°C (Vist, M. R. and J. H. Davis, 1990), the data of Fig. 5 for this cholesterol concentration can be used to determine membrane thickness as a function of temperature.

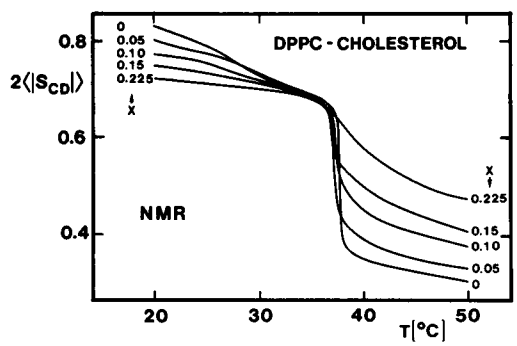

FIGURE 5 NMR order parameter, $2\langle|S_{CD}|\rangle$ cf. Eqs. 4 and 11 as derived using experimental data for the first moment, M_1, of the quadrupolar magnetic resonance spectrum of d_{62}-DPPC bilayers containing cholesterol (Vist, 1984). The order parameter is shown as a function of temperature for selected cholesterol concentrations, x.

DISCUSSION

In this paper we have examined the possible relationships between different thermomechanic properties of lipid bilayers using a simple model. It is important to understand such relations and their range of validity since they can be useful for indirect determinations of some membrane properties which are difficult to measure by direct methods. An example of such a property is the hydrophobic thickness which is believed to be involved in regulating certain membrane functions, e.g., via the hydrophobic matching condition for lipid-protein interactions (Mouritsen and Bloom, 1984; Sperotto and Mouritsen, 1988). We have pointed out that experimental measurements of ^{2}H-NMR order parameters and the first moment of the quadrupolar resonance line splitting provide an experimentally simple and feasible way of estimating membrane hydrophobic thickness. This approach is not directly applicable to phases exhibiting chain tilt ($L_{\beta'}$) or ripples ($P_{\beta'}$) since these phases do not fulfill the assumption of axially symmetric averaging of the chain motions about the bilayer normal. Even though this assumption does not hold for the low-temperature phases of e.g., pure phosphatidylcholines, it may well do so for mixtures of phosphatidylcholines and other membrane components, such as cholesterol and proteins, which tend to remove the chain tilt at low temperatures (Chapman et al., 1979; Hui and He, 1983; Presti, 1985). Hence, the present work may be particularly advantageous for two- and many-component membranes for which direct thickness measurements by diffraction techniques are not available. Furthermore, these ^{2}H-NMR techniques are not affected by the well-known difficulties associated with the procedure of deriving hydrophobic thicknesses from diffraction studies of lamellar repeat distances which invariably involves estimates of thicknesses of the interlamellar water layer and the polar-head group region (Zaccai et al., 1979; Lis et al., 1982*a*, *b*; Lewis and Engelman, 1983; Cornell and Separovic, 1983). As a noninvasive technique, the ^{2}H-NMR approach also avoids the problems with bilayer stability and the unavoidable effects of solvents present in classical capacitance and optical reflectance measurements of bilayer thickness (Dilger et al., 1982).

We have illustrated the potential of the approach by considering a particular binary membrane system, the DPPC-cholesterol bilayer. For this system, model calculations provide theoretical estimates of both the hydrophobic membrane thickness, d in Fig. 3, and the acyl-chain order parameter, S in Fig. 4. The theoretical model, on which the calculations are based, is a microscopic interaction model which takes due account of both cholesterol's crystal-breaking properties and its ability to order the lipid acyl chain. This model is the first one (cf. Ipsen et al., 1987) able to reproduce the essential features of the phase diagram, Fig. 1. By working at the level of individual acyl-chain conformational states, their statistics and mutual interactions, the present extended version of the microscopic interaction model has for the first time provided theoretical predictions for the variation of membrane thermomechanic properties with both temperature and cholesterol concentration. Within this model, the orientational order parameter, Fig. 4, and the hydrophobic thickness are linearly related, cf. Eq. 9. One of the drawbacks of the model is that it is formulated on a lattice and hence neglects aspects of the molecular packing properties. Specifically, the model does not account for effects due to the additional volume which becomes available, underneath the shorter cholesterol molecule, to the methyl terminal of the acyl chains. This in turn reflects the fact that the lattice model does not deal seriously with lipid membrane volume, cf. Eq. 5, and how it is affected by mixing in a second component. Nevertheless, the overall good qualitative accordance between the experimentally and theoretically predicted phase equilibria suggests that the present model contains the dominant effects of the lipid-cholesterol interactions.

Considering the shortcomings of the theoretical model, the theoretical predictions for the membrane thermomechanic properties compare favorably with the available experimental measurements. The predicted behavior of the membrane area in Fig. 2 is quite similar to that measured for DMPC-cholesterol bilayers by micromechanic experiments (Needham et al., 1988) (no data is available for the DPPC-cholesterol system). It should be noted, however, that the micromechanic measurements do not yield absolute area values but only fractional changes and that only a qualitative comparison is therefore possible.

By comparing the theoretical data for the orientational order parameter in Fig. 4 with the experimental data in Fig. 5 we find (excepting effects at low temperatures due to the pretransition in the experimental system) that there is a very good quantitative agreement as to the different effects cholesterol has on the chain ordering at the two sides of the pure bilayer transition temperature. It is obvious, however, that the experimental data and the theoretical results are not in detailed quantitative agreement concerning e.g., the rate of ordering by cholesterol incorporation at higher temperatures and the lack of sharp features in the experimental data at the boundaries of the ld-lo phase. Some of these discrepancies may be attributed to deviations in the phase diagrams as well as shortcomings of the mean-field approximation.

A quantitative comparison between our theoretical predictions of hydrophobic thicknesses of membranes containing cholesterol and corresponding thicknesses

obtained from x-ray diffraction on membranes in the fluid phase is not possible at present. The reason for this is that most x-ray data refer to lamellar repeat distances (Hui and He, 1983) or is only available for pure lipid bilayers (Lewis and Engelman, 1983).

It should be remarked that we have in the present paper focused on fully hydrated bilayers of saturated acyl chains. Care must be taken in applying our results to nonsaturated chains for which the relation in Eq. 3 will not hold strictly due to alternating signs of the $S_{CD}^{(i)}$ caused by particular geometrical constraints associated with the double bonds. Any application to ^2H NMR spectra of unsaturated chains or other molecules having geometrical properties different from saturated acyl chains would require a generalization of Eqs. 3 and 9.

It would be very helpful at this stage to have a critical experimental test of the predicted linear relationship between d and $\langle|S_{CD}|\rangle$ (see Eqs. 3, 10, and 11) and of the values of the parameters α and β associated with this relationship. A useful molecule for such a test would be POPC-d_{31} (1-palmitoyl-2-oleoyl-phosphatidylcholine, where the palmitoyl chain is perdeuterated). Lipid bilayers of POPC are in the L_α phase over a wide temperature range above 0°C. NMR-measurements (M. Lafleur, P. R. Cullis, and M. Bloom, unpublished results) of mixtures of POPC-d_{31} and cholesterol have shown that $\langle|S_{CD}|\rangle$ varies by more than a factor of two between 0° and 70°C as the cholesterol concentration is varied from 0 to 45%. For such a range of $\langle|S_{CD}|\rangle$ values, Eq. 3 predicts a variation of d by ~25%. It should be possible to measure d with sufficient accuracy using x-ray diffraction to check the accuracy of the predictions of the lattice model for α and β.

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