Evidence for partial rotational order in gel phase DPPC

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ABSTRACT It is shown how the dichroic ratio of the symmetric methylene stretching modes depends upon both the rotational order of hydrocarbon chains about their long axis and the tilting of the long chains with respect to the bilayer normal. Use of a recent determination of the tilt angle from x-ray measurements together with recent dichroic infrared data yields a rotational order parameter $g = -0.30$ compared to $g = 0$ for complete disorder and $g = \pm 1$ for complete order. The negative value of g corresponds to a preference for the plane defined by the chain carbons to be more perpendicular than parallel to the plane defined by the tilt direction and the bilayer normal.

INTRODUCTION

Lipid bilayers exhibit several kinds of order/disorder. These include trans/gauche isomerization order/disorder that is primarily responsible for the main chain melting transition (1). Also included is tilt order in phases such as the $L_{\beta'}$ gel phase where the hydrocarbon chains are basically all-trans. In crystalline hydrocarbon and lipid phases (2, 3, 4) there is also rotational order of the azimuthal angle of hydrocarbon chains relative to their long axis. Such rotational ordering is natural because the shape of hydrocarbon chains is not cylindrical.

Fully hydrated lipid bilayers in the subgel phase are thought to have rotational ordering of the hydrocarbon chains (5) because, like crystalline hydrocarbon phases, there are many wide-angle x-ray peaks (6). When the temperature is raised into the gel phase of DPPC, the number of wide-angle peaks decreases and scattering is confined to the $(\pm 2, 0)$ and $(\pm 1, \pm 1)$ Bragg rods. This scattering requires a high degree of parallel packing of the hydrocarbon chains and analysis of this scattering leads directly to chain tilt angles θ_{tilt} (7). The absence of scattering along other Bragg rods in the gel phase has usually been taken as evidence suggesting, but not proving, that there is no rotational order, so the hydrocarbon chains are usually modelled as cylinders. This conclusion is probably too strong. Even if the transition from subgel to gel primarily involves rotational disordering, the existence of a nonzero tilt angle in DPPC together with the fact of noncylindrical chains leads to the expectation of partial, or liquid-like, rotational ordering in the gel phase.

Although the wide angle x-ray scattering data for DPPC provide a direct and unequivocal measure of chain tilt, these data are insensitive to the shape of the hydrocarbon chains and the rotational order parameter, g. The first purpose of this note is to show that the dichroic ratio R for the symmetrical CH_2 infrared stretching band is sensitive to g, as well as to the tilt order parameter, f. The second purpose of this paper is to use determinations of f from our recent x-ray study (7) and recent polarized attenuated total reflection infrared determinations of R by Hubner and Mantsch (8) to estimate the amount of rotational order.

THEORY

Fig. 1 describes the instantaneous orientation of an all-trans hydrocarbon chain with respect to the normal to the bilayer. The direction of the dipole moment for symmetric CH_2 stretching modes is given by

$$\begin{aligned}\mathbf{P} = \mathbf{z}&[-\cos(\gamma)\sin(\theta_t)]\\ &+ \mathbf{x}[\cos(\gamma)\cos(\phi_t)\cos(\theta_t) - \sin(\gamma)\sin(\phi_t)]\\ &+ \mathbf{y}[\cos(\gamma)\sin(\phi_t)\cos(\theta_t) + \sin(\gamma)\cos(\phi_t)]. \quad (1)\end{aligned}$$

The usual order parameter for tilt is

$$f = \langle 3\cos^2\theta_t - 1\rangle/2. \quad (2)$$

The order parameter corresponding to rotation γ in a plane is

$$g = \langle 2\cos^2\gamma - 1\rangle. \quad (3)$$

The relative absorbances for the electric field in the three principle directions are obtained from the squares of the components in Eq. 1. However, even oriented bilayers are disordered in-plane, so all values of the angle ϕ_t are equally probable and it is easily integrated over. This yields

$$k_x = k_y = ({}^1\!/\!{}_2)\langle\sin^2\gamma + \cos^2\gamma\cos^2\theta_t\rangle \quad (4)$$

and

$$k_z = \langle\cos^2\gamma\sin^2\theta_t\rangle.$$

Although it has been allowed that γ is coupled to the direction of tilt, ϕ_t, it will now be assumed that γ is not correlated with the magnitude of tilt, θ_t. Then, averages over γ and θ_t in Eq. 4 may be performed independently with the result

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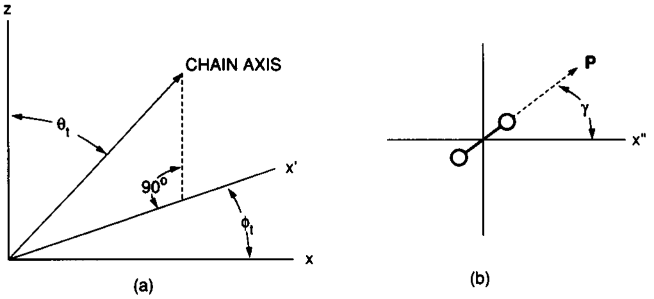

FIGURE 1 Orientation of an all-trans hydrocarbon chain. (*a*) The chain axis is tilted by θ_t from the bilayer normal which is along the *z*-axis. The *x'* axis is the intersection of the *xy* plane with the tilt plane defined by the directions of the chain axis and the *z*-axis. (*b*) View down the chain axis with the zig-zag hydrocarbon chain represented by two circles. The *x''* axis is in the tilt plane. **P** is the direction of the transition dipole moment which is perpendicular to the chain axis and is rotated by γ from *x''*.

$$k_y = (1/4)[(1-g) + (1+g)(2f+1)/3] \qquad (5)$$

and

$$k_z = (1+g)(1-f)/3.$$

Hubner and Mantsch (8) employ a 45° angle of incidence and a germanium substrate with index of refraction 4 compared to 1.44 for the lipid film. Under these conditions their appendix (8) yields the first part of the following equation and the second part follows from Eq. 5:

$$\begin{aligned} R_{HM} &= 0.85 + 1.15k_z/k_y \\ &= 0.85 + 2.30(1+g)(1-f)/(2-g+f+fg). \qquad (6) \end{aligned}$$

Eq. 6 emphasizes that R_{HM} depends upon both tilt angle θ_t through f and rotation angle γ through g. It is convenient to define

$$R_1 = (R_{HM} - 0.85)/2.30. \qquad (7)$$

Then, solving for f yields

$$f = [1 + g - R_1(2-g)]/[(1+g)(1+R_1)]. \qquad (8)$$

Assuming $g = 0$ in Eq. 8 yields Eq. A7 given by Hubner and Mantsch (8), which they derived with this explicit assumption. Solving Eq. 6 for g yields

$$g = [-1 + R_1(2+f)/(1-f)]/(1+R_1). \qquad (9)$$

RESULTS AND DISCUSSION

Our recent x-ray results yield $\theta_t = 32°$ ($f = 0.579$) for more than 1000 fully hydrated DPPC bilayers oriented on a glass slide at 19°C (7). For similar samples, though on germanium, Hubner and Mantsch (8) quote $R_{HM} = 1.1$ for the symmetric CH_2 stretch bands in the gel phase and their data in Fig. 6 for the temperature range 23–29°C are consistent with this value at 19°C. Eq. 9 then yields $g = -0.30$ which would correspond to $\gamma = 53.8°$ if the distribution were a delta function at one angle.

It is difficult to obtain perfectly oriented bilayers and a mosaic spread θ_{mo} in the normals to the bilayer will alter the value of f that is required in Eq. 9 by

$$f = (1/2)\langle 3\cos^2\theta_{mo} - 1)\rangle(1/2)\langle 3\cos^2\theta_t - 1)\rangle. \qquad (10)$$

Although Hubner and Mantsch (8) did not report a mosaic spread, our mosaic spread (7) was of the order of 5–10°. This would decrease f to 0.553 and change g to −0.34. However, this only increases γ to 54.9°.

As temperature increases from 19 to 29°C it appears (7) that θ_t may decrease about a degree and this would change g to −0.26 assuming that the dichroic ratio R remains unchanged at 1.1. This relative change in g is consistent with decreasing partial order with increasing temperature as would be expected.

It may be mentioned that the rotational order parameter g does not appear in the formula for the dichroic ratio if the transition dipole is along the chain axis and then Eq. A6 in reference 8 applies. This makes it possible, in theory, to determine f from the wagging bands, and then to determine g from the symmetric CH_2 stretch bands. Hubner and Mantsch (8) report only that R_{HM} for the wagging bands is greater than 8, which only yields $\theta_t < 29.6°$. One possibility is that their θ_t is close to 26° for their samples, which is then simply inexplicably different from the $\theta_t = 32°$ for our samples (7). If so, then it would be possible for $g = 0$ in Eq. 9 and for there to be zero rotational order. However, if the large value of R_{HM} for the wagging bands required by their geometry precludes accurate determination, then it is suggested that the combination of θ_t from x-ray scattering and R_{HM} for the symmetric CH_2 bands may be the best way to obtain information about rotational order/disorder in the gel phase.

The small value of the rotational order parameter, $g = -0.30$, is consistent with the usual picture of the gel phase in which the isomerically ordered hydrocarbon chains are largely rotationally disordered. However, to address more subtle questions, such as whether the chains tilt toward nearest neighbors or towards next nearest neighbors, the existence of even a small amount of rotational order suggests that it might be necessary to model the chains with less symmetry than cylinders. It must also be noted that a small value of the rotational order parameter is consistent with relatively little disorder if there is more than one population of hydrocarbon chains, each with a substantial value of g, but with a sum that happens to be small, although this appears unlikely in view of the small number of wide-angle Bragg rods observed by x-ray scattering. In any case, the major point of this paper is that the combined use of x-ray scattering and infrared spectroscopy provide information about a previously inaccessible property, partial rotational order of hydrocarbon chains in gel phase lipid bilayers.

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