DETERMINING BILAYER HYDROCARBON THICKNESS FROM NEUTRON DIFFRACTION MEASUREMENTS USING STRIP-FUNCTION MODELS

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ABSTRACT Neutron diffraction methods provide information about the distribution of matter in biological and model membrane systems. The information is derived from plots (profiles) of scattering length density along an axis normal to the membrane plane. Without the use of specific deuteration, the generally low resolution of the profiles limits their interpretation in terms of specific chemical constituents (e.g., lipid headgroup, lipid hydrocarbon, protein, and water). A fundamental and useful structural assignment to make is the boundary between the headgroup and hydrocarbon regions of bilayers. We demonstrate here that strip-function model representations of neutron scattering length density profiles of bilayers are sufficient to determine accurately the position of the headgroup-hydrocarbon boundary. The resulting hydrocarbon thickness of the bilayer is useful for determining the area per lipid molecule and consequently the molecular packing arrangements of the membrane constituents. We analyze data obtained from dioleoylphosphatidylcholine (DOPC) bilayers at 66% RH using standard Fourier profile analyses and from DOPC deuterated specifically at the C-2 carbon of the acyl chains using difference Fourier analysis. We demonstrate that strip-function models accurately define the positions of the C-2 carbons and thus the hydrocarbon thickness (d_{hc}) of the bilayer. We then show, using quasi-molecular models, that the strip-model analysis probably provides an accurate measure of d_{hc} because of the exceptionally high scattering length density difference between the carbonyl and methylene groups.

INTRODUCTION

Neutron scattering length density profiles normal to the membrane plane can easily be obtained by Fourier transformation of lamellar neutron diffraction patterns (see reviews by Worcester, 1975; Franks and Levine, 1981). The question of the proper decomposition of these profiles into "headgroup" and "hydrocarbon" regions is an important one that has relevance to many questions in membrane biophysics. For example, the variation of hydrocarbon thickness vs. chain length (Lewis and Engelman, 1983), the extent of localization of water in the headgroup region (Simon et al., 1982; Zaccai et al., 1975), and the packing densities of the hydrocarbon region of the bilayer (King et al., 1985), are all questions which rely on a correct determination of the headgroup-hydrocarbon boundary. The area/lipid is an important parameter that can be obtained if this boundary is determined and the average mass density of the hydrocarbon core is known. It appears that one can assume the density to be that expected of bulk hydrocarbons (Levine and Wilkins, 1971) if there are no "solutes" dissolved in the region (King et al., 1985).

The hydrocarbon region of bilayers formed from ester-linked phospholipids is generally taken as the acyl chains excluding the carbonyl groups (Andrews et al., 1970; Fettiplace et al., 1971; White, 1978; Lewis and Engelman, 1983). Thus, the acyl chain C-2 carbons define the inclusive extent of the hydrocarbon region to which we assign the thickness d_{hc}. If the headgroups and C-2 methylene groups were lined up in neat consecutive rows, the hydrocarbon core structural boundary, and thus d_{hc}, would be clearly defined. The boundary is conceptually less clear, however, for a disordered liquid-crystalline bilayer. It is not unreasonable to expect the averaged positions of the C-2 carbons to define the boundary such that for every C-2 carbon located a certain distance outside the average, another will be found the same distance inside the average. In lieu of a rigorous statistical mechanical model definition, we describe a phenomenological approach which we feel adequately describes this boundary with respect to biophysical questions like those described above.

Strip-function models (Worthington, 1969) have proven to be a very helpful method of representing the profile structures of biological membranes. We describe in this paper the use of such models for defining d_{hc}. While strip-function models cannot generally be considered precise models of membrane scattering length density profiles, they are excellent representations of the average scattering length densities and widths of various regions of the profiles. They are thus particularly appropriate for disordered systems like liquid-crystalline lipid bilayers because they make the fewest number of assumptions about the

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structural arrangement. Other kinds of model representations of the bilayer structure such as molecular models (Franks, 1976) and Gaussian function models (Mitsui, 1978) are also useful in defining membrane structure and can take into account thermal disorder. However, they often require more experimental parameters for a unique fit than are available.

We proceed as follows. First, we present a standard analysis of eight orders of diffraction data from DOPC at 66% RH that includes the construction of standard neutron scattering length density profiles. Second, we analyze data obtained from DOPC deuterated specifically at the C-2 carbon which accurately define the headgroup-hydrocarbon boundary. Third, we discuss the construction of strip-function models and derive one that satisfies our data for DOPC and predicts within experimental error the locations of the C-2 carbons. Fourth, we use a quasi-molecular model of liquid-crystalline DOPC whose structure factors agree with the experimentally determined factors to demonstrate that the strip models probably work because of the "high contrast" scattering length density boundary between the carbonyl and methylene groups.

MATERIALS AND METHODS

Sample Preparation

DOPC was purchased from Avanti Polar-Lipids, Inc. (Birmingham, AL) and checked for purity using thin layer chromatography. DOPC specifically deuterated at the C-2 carbons of the acyl chains was a gift from Prof. J. Seelig. Multilayers were deposited on a quartz slide by evaporation from a 1:1 chloroform/methanol solution and mounted in the neutron beam in a sealed and thermostated aluminum chamber containing a reservoir of a saturated solution of sodium nitrite in water to maintain the relative humidity at 66% (T = 22.5°C).

Data Collection and Correction

The neutron diffraction data in the form of integrated intensities I(h) were collected on either the H-4 satellite or the H-4 beampipe at the High Flux Beam Reactor at Brookhaven National Laboratory. For the satellite experiments, a two-dimensional position-sensitive detector was used to collect the data and a step-scanning diffractometer with a single gas-filled proportional counter was used in the θ-2θ mode on the beampipe. The precision of the measurement of the unit cell repeat distance (d-spacing) was ±0.5 Å. Because the orientation of the samples was very high [mosaic spread of ~0.1° (FWHM)], it was necessary to rotate the sample in θ to obtain the complete pattern. Thus, it was also necessary to correct for the variation of the sample-beam intersection with θ. This correction was performed numerically from geometrical considerations of the shapes, cross-sectional areas, etc. of the beam and sample. In addition, an inverse Lorentz correction factor of h, where h is the diffraction order, was used for the hth diffraction peak.

The data sets from the deuterated DOPC were scaled to the protonated sets using the structure factors of the water layer established from measurements made at the same total water content using H_2O-D_2O exchange as described by Büldt et al. (1979). The complete data sets of either protonated or deuterated DOPC were invariably derived from measurements on several different samples. Each set (i) from the several samples were scaled to each other by establishing a scale factor $k(i)$ relative to a reference (r) set that satisfies the relation $k(i) = \Sigma F_r^2(h)/\Sigma F_i^2(h)$.

The corrected observed intensities of each diffracted signal, $I_{obs}(h)$, were used for the analyses described in this paper. We note that, properly, h ranges from zero to H where H is the value of the highest h observed. The zeroth order term is related to the absolute scattering length density or mass density of the sample but cannot be observed under ordinary circumstances so that, practically, h ranges from 1 to H. The importance of $I_{obs}(0)$ and methods of obtaining it for absolute scale analyses have been discussed by King et al. (1985).

Data Analysis

The structure factors for the analyses were obtained with a precision of ±2% (orders one through four) or ±10–15% (orders five through eight) from the observed intensities using the relation

$$F_{obs}(h) = S(h)\sqrt{I_{obs}(h)},$$

where $S(h)$ is a phase factor that must be determined through additional experiments. The phase angles for centrosymmetric structures are either 0 or 180° so that $S(h) = \pm 1$. Phases were determined by analysis of H_2O-D_2O exchange experiments and by swelling experiments as described elsewhere (King et al., 1982). The strip-function analysis described elsewhere in this paper was not used in any way to determine phases.

The standard method for analyzing bilayer structures is to construct scattering length density profiles, $\rho_{rel}(z)$, on an arbitrary relative scale by means of a Fourier transformation of the $F_{obs}(h)$. The scale is arbitrarily determined by detector counting times, geometry, etc. $\rho_{rel}(z)$ is given by

$$\rho_{rel}(z) = \frac{2}{d}\sum_{1}^{H} F_{obs}(h)\cos(2\pi hz/d), \quad (1)$$

where d is the Bragg spacing (d-spacing) and z is the coordinate normal to the plane of the membrane. We found d = 49.7 ±0.5Å. The general methods for analyzing the data to obtain neutron scattering length density profiles and strip-function representations of the profiles on absolute and relative scales have been described elsewhere (King et al., 1982; 1985).

The calculations described in this paper require that the scattering lengths (B_i) of various "parts" of DOPC be known. These are arrived at by summing up the known individual scattering lengths of each atom in the "part." A list of these is given by Schoenborn (1975).

The locations of the specifically deuterated C-2 methylenes were determined using difference Fourier structural analysis as described elsewhere (Büldt et al., 1978; Büldt et al., 1979; White et al., 1981; King et al., 1985; White and King, 1985). In brief, a "difference structure" in real space can be determined from the so-called difference structure factors (i.e., reciprocal space) obtained by subtracting the structure factors of protonated DOPC from those of deuterated DOPC. The real space difference structure shows the location of the specifically deuterated groups.

RESULTS AND DISCUSSION

Profile Analysis of DOPC Bilayers at 66% RH

The structure factors for DOPC [$F_{obs}(h)$] and for DOPC specifically deuterated at the C-2 position of the acyl chains [$F_D(h)$] are shown in Table I along with the structure factors $F_{strip}(h)$ and $F_{mod}(h)$ calculated for the strip-function and quasi-molecular models. Scattering length density profiles for the nondeuterated DOPC on a relative scale are shown in Fig. 1. The data for the specifically deuterated DOPC and the models will be discussed later. Profiles for DOPC were constructed using the first four diffraction orders alone (dashed curve) or

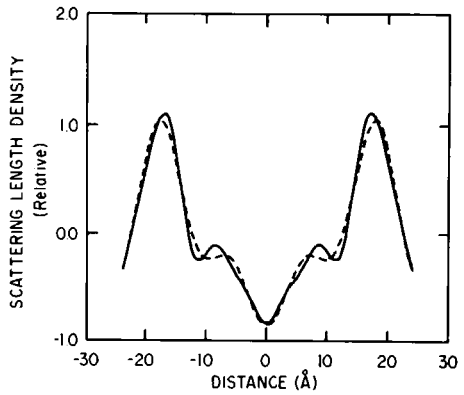

FIGURE 1 Neutron scattering length density profiles ($\rho_{rel}[z]$) of DOPC at 66% RH and 22.5°C. The profiles are Fourier transforms (Eq. 1) of the $F_{obs}(h)$ for nondeuterated DOPC (Table I).The solid curve is the Fourier synthesis using all eight orders of data and the dashed curve the synthesis using only the first four $F_{obs}(h)$ (h = 1 to 4). The central trough can be attributed to the acyl chain terminal methyl groups, which have smaller scattering lengths than the methylenes due to the extra hydrogen. The peaks adjacent to the trough can be attributed to the double bonds, which have a larger scattering length than adjacent methylenes because of fewer hydrogens. The larger peaks beyond the double bond peaks represent the headgroup.

using the entire eight orders (solid curve). The data have been plotted in this way because in many experiments it is not practical to measure more than four diffraction orders due to small signal strengths. It is thus useful to compare the profiles at the two resolutions. Both curves show two pairs of peaks and a central trough. The large peaks are associated with the polar groups and water whereas the small ones immediately adjacent to the trough are due to the double bonds on the acyl chains based upon specific deuteration experiments (unpublished results). The lack of hydrogens on the double bonds cause them to scatter strongly relative to the methylenes and thus be a prominent feature of the acyl chains. The trough is due to the terminal methyl groups which, scatter relatively less than the methylenes because of the extra hydrogen. Comparison of the two curves shows that the peaks of the four-order curves are shifted relative to the peaks of the eight-order curve. Such shifts are common in low-resolution profiles and one must thus be careful when using limited resolution real-space profiles to obtain distance information.

The profiles do not provide any immediately obvious clues as to the position of the boundary between the polar groups and the hydrocarbon core of the bilayer. Strip-function representations of the profiles, however, seem to provide a well-defined boundary. Before describing the derivation of these representations, we first describe the results of the measurements on DOPC deuterated specifically at the C-2 carbons, which unambiguously define the extent of the hydrocarbon region.

Specifically Deuterated DOPC

The data of Table I for protonated DOPC and DOPC deuterated specifically at the C-2 carbons of the acyl

TABLE I
OBSERVED AND CALCULATED STRUCTURE FACTORS FOR DOPC BILAYERS AT 66% RH AND 22.5°C

h	$F_{obs}(h)$	$F_{strip}(h)$	$F_{mod}(h)$	$F_D(h)$
1	−68.0 ± 1.4	−68.0	−67.7	−87.8 ± 1.8
2	−38.3 ± 0.8	−38.3	−35.7	−86.9 ± 1.7
3	40.9 ± 0.8	40.9	41.1	61.3 ± 1.2
4	−44.0 ± 0.9	−47.9	−46.3	−19.1 ± 0.4
5	−5.0 ± 0.5	−5.2	−5.6	−9.9 ± 1
6	7.1 ± 0.7	7.0	8.0	0.0
7	0.0	1.0	0.1	3.0 ± 0.3
8	−8.0 ± 0.9	−1.3	−7.6	−1.6 ± 0.2

$F_{obs}(h)$ are the experimentally determined structure factors of nondeuterated DOPC and $F_D(h)$ for DOPC specifically deuterated at the C-2 carbons of the acyl chains. $F_{strip}(h)$ are for the strip-function model and $F_{mod}(h)$ for the Gaussian quasi-molecular model. See text for details.

chains were used to construct a difference profile on the relative scale. The structure factors $F_{obs}(h)$ were subtracted from the $F_D(h)$ to yield difference-structure factors from which the real-space structure shown in Fig. 2 (dashed curve) was constructed by Fourier synthesis. It is not unusual for the peaks to be slightly skewed as a result of accumulative experimental error and termination errors. Therefore, we fitted a Gaussian peak to the data in reciprocal space in the manner of Büldt et al. (1979) and Zaccai et al. (1979) to determine the mean position and distribution of the C-2 carbons. The result of the fitting procedure is shown in real space in Fig. 2 (solid curve). The peak to peak distance of the C-2 carbons across the bilayer determined in this way is 27.6 ±0.5Å.

Strip-Function Models

One of the first questions to arise when constructing strip-function models is the one of how many strips are necessary to represent the structure at the given resolution limit. We have found the following simple guidelines to be useful. First of all, visual inspection of the limited resolution Fourier synthesis is obviously helpful. For example, from the syntheses in Fig. 1, it is apparent that separate strips representing the central methyl trough region, the methylene/double bond region, the headgroup region and the water region, respectively, are reasonable choices. Second, an attempt should be made to assure that the widths of the various regions are on the order of or larger than the resolution limit, $d/2H$ (Blaurock and Worthington, 1966) where d is the unit cell size (Bragg spacing) and H is the highest observed diffraction order. Finally, trial and error testing is useful for picking the minimum number of strips that still give reasonable agreement with the observed structure factors. There is a simple relationship between the number (s) of unique strips chosen and the minimum number (j) of parameters necessary to specify uniquely the strip model of a centrosymmetric structure. Each strip is specified by a width and scattering length

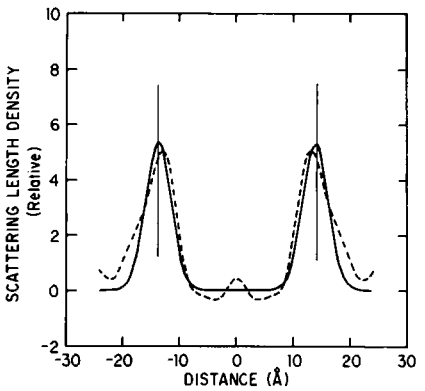

FIGURE 2 The difference structure (dashed curve) for DOPC specifically deuterated at the C-2 carbons of acyl chains. It is obtained by Fourier synthesis from the difference structure factors given by $F_D(h) - F_{obs}(h)$ from Table I. Because the deuterated C-2 peak is slightly skewed, a Gaussian curve (solid line) has been fitted to it in reciprocal space to determine the mean position. The vertical lines show the position of the boundary of the hydrocarbon region as determined by the eight-order strip-function model (Table II). The agreement is excellent and indicates that $2Z_2$ (Fig. 3) is a good measure of the thickness d_{hc} of the hydrocarbon core of the bilayer.

density. However, if the analysis is done on a relative scale, one of the scattering length densities is arbitrary (Worthington, 1969). Because the d spacing is known, $j = 2s - 2$.

These guidelines lead us to consider strip-function models for DOPC of the type shown in Fig. 3. Noting the centrosymmetric nature of the model and that the central trough can be considered as two side-by-side strips, there are $s = 4$ unique strips to be described by $2s = 8$ parameters. They are the methyl trough scattering length density (ρ_1) and half-width (Z_1), the methylene/double bond density (ρ_2) and width ($Z_2 - Z_1$), the headgroup density (ρ_3) and width ($Z_3 - Z_2$), and a water layer of density (ρ_4) and width ($Z_4 - Z_3$). Each strip must in

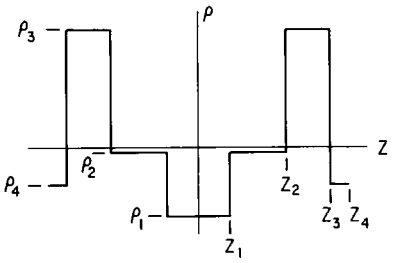

FIGURE 3 Strip-function model parameters. The number of strips chosen to represent the average scattering length densities of various regions of the bilayer is based in part upon the resolution of experiment given by $d/2H$, where H is the number of diffraction orders observed. Z_1 and ρ_1 are the boundary and average scattering length density of the methyl trough region, Z_2 and ρ_2 the methylene/double bond region, and Z_3 and ρ_3 the headgroup region. ρ_4 is the average scattering length density of the water region. We show in this paper that $2Z_2$ is an accurate measure of the hydrocarbon thickness of the bilayer, d_{hc}. (The strip-parameters used in constructing this particular curve correspond to the four-order parameters of Table II.)

reality contain an admixture of adjacent regions so that a strip width essentially defines reasonable bounds between which the major components spend most of their time on the average. The minimum number of parameters necessary to define the model will be $j = 8 - 2 = 6$ defining $2Z_4 = d$ and letting ρ_4 be arbitrary (relative scale). We derive strip-model parameters below using eight orders and four orders of diffraction data. In the latter case, there are fewer measured parameters than the minimum number of parameters necessary to specify the the model; the method of specifying the additional parameters will be described later.

The goal of the analysis is to choose values of the parameters that lead to strip-function structure factors, $F_{strip}(h)$, which agree within experimental errors (shown in Table I) to the actual structure factors $F_{obs}(h)$. King (1971) has shown the calculated structure factors for this model, for $h = 0$, to be

$$F_{strip}(h) = \frac{d}{\pi h}\sum_{i=1}^{n-1}(\rho_i - \rho_{i+1})\sin(2\pi h z_i/d), \qquad (2)$$

where z_i is the position of the ith boundary and ρ_i is the scattering length density of the ith region. These $F_{strip}(h)$ are compared with the $F_{obs}(h)$ via the so-called R-value

$$R = \frac{\sum_h \| F_{strip}(h)| - K'|F_{obs}(h)\|}{\sum_h K'|F_{obs}(h)|}, \qquad (3)$$

where K' is a scaling factor such that

$$K' = \left[\frac{\sum_h |F_{strip}(h)|^2}{\sum_h |F_{obs}(h)|^2}\right]^{1/2}. \qquad (4)$$

The procedure we use for determining the proper values of the parameters is to search exhaustively by computer for a minimum of the R-value by a brute force direct search technique. Briefly, an initial set of starting parameters are chosen on a large net and the net size is systematically reduced in the search for a minimum. Through multiple runs with different starting parameters and different net sizes one can ensure that all of the minima (if more than one) can be found. Of course, the analysis is only useful for cases where a unique global minimum is obtained. Even though the problem is underdetermined if the number (H) of structure factors is smaller than the number of parameters specifying the model, we have observed that a unique minimum for one of the parameters can sometimes be found even when $H \neq j$. This is the case for DOPC and we show below a strip-function model with a uniquely determined hydrocarbon thickness derived using four orders of data, which gives essentially the same value as the model based upon eight orders of data. We do not know if it will be generally true

TABLE II
SUMMARY OF PARAMETERS FOR STRIP-FUNCTION MODELS DERIVED USING FOUR ORDERS OR EIGHT ORDERS OF DATA FROM THE $F_{obs}(h)$ OR $F_{mod}(h)$ OF TABLE I

	$F_{obs}(h)$		$F_{mod}(h)$	
	Four orders	Eight orders	Four orders	Eight orders
ρ_1	−0.57	−0.70	−0.58	−0.67
ρ_2	−0.06	−0.12	−0.10	−0.14
ρ_3	0.96	1.03	0.92	1.02
ρ_4	−0.41	−0.11	−0.42	−0.09
Z_1	5.70	3.60	5.70	3.50
Z_2	14.20	13.90	14.20	13.90
Z_3	22.10	21.10	22.10	21.00
Z_4	24.85	24.85	24.85	24.85

The parameters are defined in Fig. 2. The distances Z_i have units of Angstroms. The scattering length densities ρ_i are in relative units corrected in such a way that zero corresponds to true zero scattering length density (see King et al., 1985).

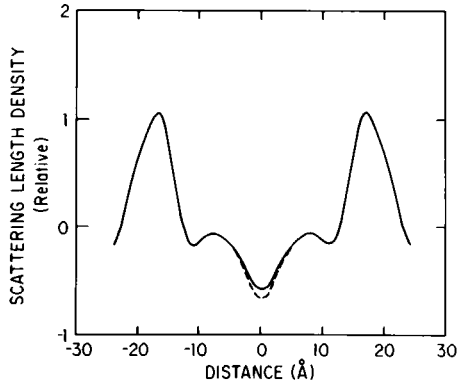

FIGURE 5 Quasi-molecular model representations of the DOPC bilayer neutron scattering length density profile. The solid curve is the profile obtained by summing the curves shown in Fig. 4. The dashed curve is the profile obtained from Eq. 1 using the first eight of the structure factors ($F_{mod}[h]$) for the model (Table I). The dashed curve's greater "depth" at the center is a result of Fourier termination error referred to as Gibbs' phenomenon (Gibbs, 1898).

that a unique minimum can be found when $H \neq j$. There may be particular combinations of H and j that give unique minima. Without knowing what the combinations might be, the question of uniqueness remains a matter for empirical verification.

Table II shows the strip-model parameters based on the $F_{obs}(h)$ of Table I for nondeuterated DOPC. Also included are the parameters based on the $F_{mod}(h)$ for the quasi-molecular model described later. The strip-functions shown in Figs. 3 and 5 use the four-order and eight-order data, respectively. Of primary concern here is the width $2Z_2$, which we presume at this point to be the width of the hydrocarbon core. Also of interest is the comparison of the values of $2Z_2$ found from the four-order (28.4 Å) and eight-order (27.8 Å) models. We estimate the precision of these numbers to be about ±0.5 Å, based on experimental errors and the uncertainties involved in the fits of the models to the data. The two widths are in reasonable agreement with one another and with the transbilayer distance between the C-2 carbons of 27.6 ±0.5 Å reported in the previous section.

The eight-order model is unique because $H = 8$ is greater than $j = 6$. To arrive at the four-order model, we had to make assumptions about two of the six parameters. To avoid arbitrary choices, we chose $2Z_3$ to be the lipid thickness d_ℓ calculated by the method of Luzzati (1968) and $2Z_1$ to be the width of the central region of the bilayer that hexane occupies when it dissolves in DOPC (White et al., 1981). This width was believed to approximate the methyl trough width and was determined from neutron diffraction measurements on DOPC multilayers containing deuterated hexane. We found the final value of $2Z_2$ to be very insensitive to these choices as can be seen by examining the differences in Z_1 and Z_3 in the four-order and eight-order models. The boundaries of $2Z_2$ apparently dominate strongly the fit of the strip-model to the observed structure factors. It is probably important that the width of

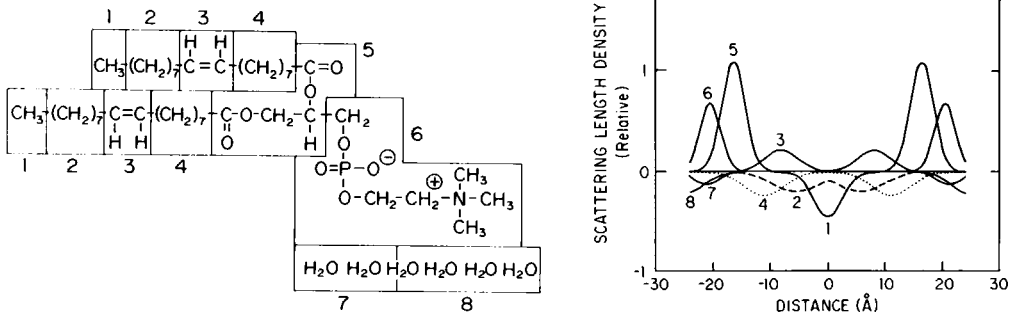

FIGURE 4 Compositions and scattering length density distributions of the "parts" of the quasi-molecular model for a DOPC bilayer. (*a*) The "parts" of DOPC assigned to each Gaussian peak is shown in *b*. The atomic compositions per lipid molecule of the various peaks are as follows: 1, $2CH_3$; 2, $14CH_2$; 3, 4CH; 4, $14CH_2$; 5, $2CO_2 + CH_2 + CH$; 6, $3CH_2 + 3CH_3 + N + PO_4$; 7, $2.5H_2O$; 8, $3.5H_2O$. The water content is based upon the data of Jendrasiak and Hasty (1974). (*b*) The superpositions of the various curves representing the different "parts" of the molecule. Each peak is a Gaussian curve centered at Z_i with a half-width of A_i at $1/e$ of the maximum amplitude. The area under each peak times the area/molecule (S) is equal to the sum B_i of the scattering lengths of the atoms attributed to the peak (molecular "part").

$2Z_2$ is significantly greater than the resolution limit $d/2H$ even for $H = 4$.

We now turn our attention to the question of why the strip-function analysis so accurately predicts d_{hc}. We first describe a quasi-molecular model for the DOPC and then examine strip-models derived from it using the same criteria as the actual data. We show that the strip-models strongly select the boundary between the carbonyl groups and acyl chain methyl groups. This boundary coincides with the C-2 groups.

Strip-Function Analysis of a Quasi-Molecular Model

We have constructed a number of more or less arbitrary molecular models of bilayers with various groups in defined positions and then done strip-function modeling of these molecular models using the procedures of the previous section (see for example, White et al.,1985). We invariably find the edges of the strip corresponding to the hydrocarbon region coincide with the carbonyl/methylene boundary. Rather than use arbitrary models for describing this conclusion, we choose to use a quasi-molecular model whose structure factors agree with the ones observed for DOPC at 66% RH. The model is based on extensive measurements of the distribution of double bonds and water in the DOPC bilayers using specific deuteration. A full description of this work is too lengthy for inclusion in this paper and will thus be described in detail elsewhere.

The basic idea is to derive strip-function models for the molecular model using the same approach as for the original data. This allows us to relate molecular "parts" to the various strips. Model structures can be constructed by an appropriate "disordering" of known (Hitchcock et al., 1975) crystalline lipid structures (e.g. Franks, 1976; Worcester and Franks, 1976) or by the approximation of reasonable liquid-crystalline bilayer structures via the use of Gaussian distributions of the molecular parts (e.g., Mitsui, 1978). The latter method is particularly convenient to implement and is the one we use here. The division of the molecule into multiatomic parts provides what we call a quasi-molecular model. This approach is entirely appropriate because our resolution is limited to a value between d/H and $d/2H$. The width of a typical "part" should be, conservatively, $d/8$ for eight diffraction orders of data.

The procedure is to partition the bilayer into appropriate molecular "parts" and represent these parts by Gaussian distribution functions. Each part's Gaussian is scaled by equating its area to the summed neutron scattering lengths of the atoms constituting the "part." We partition the bilayer as shown in Fig. 4 into eight Gaussians representing the methyl groups, the methylenes below the double bond, the double bonds, the methylenes above the double bonds, the glyceryl esters, the phosphorylcholines, the interbilayer water, and the headgroup water. The widths of these Gaussians as well as six of their positions (the methyl and interbilayer water positions are already known to be at $z = 0$ and $z = d/2$, respectively) are the 14 parameters that serve to define the model structure. For one of the molecular parts whose constituent atoms have the combined neutron scattering length B_i, one can write

$$\rho_i(z) = \frac{B_i}{A_i S\sqrt{\pi}} \exp - \left(\frac{z - Z_i}{A_i}\right)^2 \quad (5)$$

for its contribution to the neutron scattering length density profile. Here, A_i is the $1/e$ half-width of the Gaussian distribution for the given component and Z_i is its position across the bilayer. S is the (unknown) area per lipid and can be assigned an arbitrary value because we are working on a relative scale (here we choose $S = 70$ Å^2). Relations similar to Eq. 5 are written for each of the molecular parts; the quasi-molecular model neutron scattering length density profile for $0 \leqq z \leqq d/2$ is simply the sum of these relations given by

$$\rho_{mod}(z) = (S\sqrt{\pi})^{-1} \sum_{i=1}^{8} (B_i/A_i) \exp - [(z - z_i)/A_i]^2. \quad (6)$$

Using a global computer search procedure similar to that used for finding the strip-function parameters, the parameters of the model are varied until the Fourier transform coefficients of the model (the model's structure factors, $F_{mod}(h)$ agree within experimental error with the observed structure factors. The model structure factors are

$$F_{mod}(h) = (2/S) \sum_{i=1}^{8} B_i \exp - (\pi A_i h/d)^2 \cos (2\pi Z_i h/d). \quad (7)$$

The Gaussian structural parameters chosen for the model are summarized in Table III. For the purposes of our discussion, one can consider this as an arbitrary model. However, we will show elsewhere that it is a correct model for DOPC at our resolution. In any case, the structure factors of the model agree within experimental error with the observed ones.

The results of the strip-function model analysis of the quasi-molecular model are shown in Table II along with the strip-model parameters from the analysis of the original data. The profile of the molecular model, obtained by summing together the curves of Fig. 4, is shown in Fig. 5. The Fourier transform (Eq. 7) of this model yields the model's structure factors ($F_{mod}[h]$), which are listed in Table I. To see the effect of termination artifacts on profiles, we constructed a profile (Eq. 1) using the first eight model structure factors. This profile is shown as the dashed curve in Fig. 5. The termination error acts to increase artificially the depth of the methyl trough. This is an example of Gibbs' phenomenon (Gibbs, 1898).

A comparison of the contents of Tables II and III shows that twice the glyceryl-ester peak position ($Z_5 = 16.4$ Å) minus its $1/e$ width ($A_5 = 2.8$ Å), 27.2 Å, is in good

TABLE III
STRUCTURAL PARAMETERS DEFINING THE QUASI-MOLECULAR MODEL OF DOPC SHOWN IN FIG. 4

Part i	Scattering length B_i	Position Z_i	Half-width A_i
1	−0.48	0.0	3.1
2	−0.70	5.5	5.8
3	0.56	8.1	3.4
4	−0.70	11.0	4.8
5	3.82	16.4	2.8
6	1.09	20.4	2.6
7	−0.45	21.1	2.8
8	−0.63	24.85	4.7

The hydrated DOPC molecule is subdivided into eight "parts." The scattering length density of each "part" as a function of distance (z) is represented by a Gaussian curve at Z_i of half-width A_i at $1/e$ of the peak amplitude. The area under each Gaussian peak equals the summed scattering lengths B_i of the atoms constituting each molecular "part." The B_i have units of 10^{-12} cm. Z_i and A_i are in Ångströms.

agreement with the model's strip-function hydrocarbon thickness parameters ($2Z_2$) of 27.8 Å ($H = 8$) and 28.4 Å ($H = 4$). This is illustrated graphically in Fig. 6 where we have superimposed the glyceryl-ester (dominated by the carbonyl groups) and phosphorylcholine peaks of the molecular model on the eight-order strip-function profile.

We conclude that the $2Z_2$ strip selects largely the acyl chains beyond the carbonyl group. The analysis strongly suggests that the strip-function method for determining d_{hc} works by selecting the boundary between the carbonyl groups and the methylene groups. Note in Table III that the strongest scattering feature (peak 5; glyceryl ester) is immediately adjacent to the weakest scattering feature (peak 4; methylenes). The $2Z_2$ strip apparently selects this "high contrast" boundary.

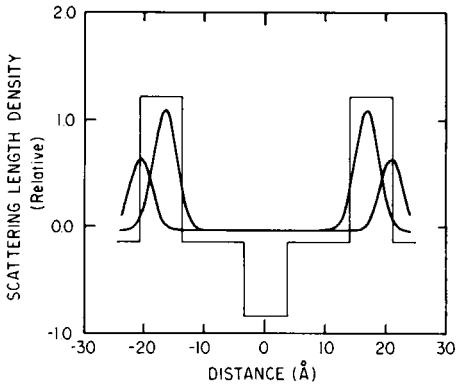

FIGURE 6 Gaussian distributions representing the glyceryl/ester and phosphorylcholine portions, respectively, of the headgroup of the quasi-molecular model (Fig. 5). Also shown is the eight-order strip-function model obtained from the structure factors $F_{mod}(h)$ (Table I). The boundary of the hydrocarbon region (Z_2, Fig. 3) coincides with Z_5-A_5 of the molecular model (Fig. 4). The strip-model effectively excludes the carbonyl group (which dominates peak 5) from the hydrocarbon core.

CONCLUSIONS

We have shown in this paper that strip-function modeling of bilayer diffraction data is a convenient and accurate method for determining the hydrocarbon thickness of lipid bilayers. The apparent reason for its success is that the glyceryl-ester group is a very strong neutron scattering feature relative to the adjacent methylene groups. The strip-function boundaries for d_{hc} are forced to be located such that they exclude the carbonyls from the methylene region. Hence, a surprisingly precise position for the carbonyl-methylene boundary can be obtained. A natural question which arises concerns the effectiveness of the method applied to x-ray rather than neutron scattering length densities. In this case, we have found that the strip-function method applied to quasi-molecular models consistently overestimates d_{hc} by 1 to 2 Å. The reason seems to be that the glyceryl-ester peak does not involve such a large change in scattering length compared with the methylenes for x rays. Alternate modeling methods for determining d_{hc} from x-ray data are being explored.

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