Relations for lipid bilayers

Connection of electron density profiles to other structural quantities

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ABSTRACT Three relations are derived that connect low angle diffraction/scattering results obtained from lipid bilayers to other structural quantities of interest. The first relates the area along the surface of the bilayer, the measured specific volume, and the zeroth order structure factor, $F(0)$. The second relates the size of the trough in the center of the electron density profile, the volume of the terminal methyl groups, and the volume of the methylene groups in the fatty acid chains. The third relates the size of the headgroup electron density peak, the volume of the headgroup, and the volumes of water and hydrocarbon in the headgroup region. These relations, which are easily modified for neutron diffraction, are useful for obtaining structural quantities from electron density profiles obtained by fitting model profiles to measured low angle x-ray intensities.

INTRODUCTION

Many low angle diffraction experiments have been performed on lipid bilayers and biomembranes in multilamellar arrays (1–10) and continuous scattering experiments have been performed on large unilamellar vesicles (11–13). By careful treatment of the phase problem electron density profiles have been produced (1–12) that conclusively establish that bilayers have electron dense headgroup regions and that non-interdigitated (8, 9) bilayers have electron sparse regions in the center where the terminal methyl groups are concentrated. (For simplicity we will henceforth explicitly mention only electron densities appropriate for x-ray studies; corresponding statements for neutron studies will be implicit.) These studies are also consistent with a region of constant electron density, occupied by the methylene groups on the fatty acid chains, between the headgroups and the terminal methyls and another region of constant electron density, occupied by water, outside the bilayers or between bilayers in multilamellar arrays.

Other measurements have focused on other structural quantities of importance for describing the organization of lipid bilayers. These include dilatometric measurements, which provide the volume per lipid molecule when hydrated (14–16), and wide-angle diffraction measurements, which provide information about chain packing (3, 4, 17–19). Information about the number of waters per lipid, n_W, required to fully hydrate multilamellar arrays is provided by hydration experiments (20, 21) and by low-angle measurements of the long spacing D as a function of added water (17, 18, 22–24); n_W has been used together with specific volume measurements to obtain the area per molecule A and many other quantities of interest (22–25), but n_W is subject to large experimental uncertainties (6, 26). The relations mentioned briefly in this paragraph have recently been systematically set forth (26).

The present paper develops three relations between the results of the research mentioned in the first paragraph above and the results mentioned in the second paragraph. The results of the low angle diffraction studies (excepting the n_w results) can be characterized by an electron density profile such as the one shown in Fig. 1. A functional form for electron density profiles that has been used extensively in the past is the strip model (5, 11, 27), which represents every region of the bilayer by a strip of constant electron density. We hasten to emphasize, however, that the relations derived in this paper are general in that they apply, not only to strip models, but to any functional form for the electron density profile which has methylene and water regions with constant electron density. Also, there is no requirement that all the water or all the methylenes must be in the regions of constant density, so these regions can be quite small, consistent with the existence of long tails on the functions representing the headgroup and the methyl regions. The particular functional form in Fig. 1 is one that we have developed (28). It represents the terminal methyls by one negative Gaussian function and represents the headgroup region by two Gaussian functions. From an electron density profile, obtained using the strip models or from our hybrid Gaussian models or from other types not yet defined, the second and third relations allow the extraction of structural information about the terminal methyl and the headgroup regions, respectively. The first relation, to which we now turn, does not even require an electron density profile, but it can utilize one if it is available.

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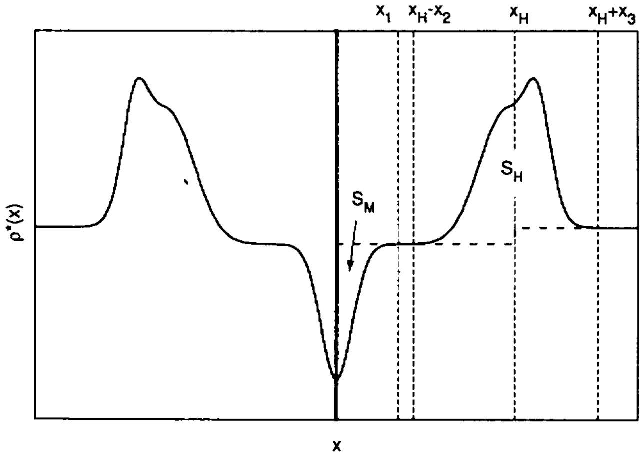

FIGURE 1 An example of an electron density profile with electron density on the vertical axis and distance x along the normal to the bilayer on the horizontal axis. The position $x = 0$ is at the center of the methyl trough in the center of the bilayer. The integrated areas, S_M and S_H, for the methyl and headgroup regions are indicated on the right-hand side of the figure.

Relation for *AF*(0)

Low angle scattering from single bilayers yields the square of the continuous structure factor, $F(X)$ (1, 11). Low angle diffraction from multilamellar arrays with repeat distance D samples the square of $F(X)$ for scattering vector $X = 2\pi h/D$ for integer values of h (1–10). Provided that the bilayers do not change shape, swelling or dehydration experiments allow $F(X)$ to be sampled at additional values of X corresponding to different D spacings (1, 2, 6, 9, 10). Use of the sampling theorem allows the construction of the same continuous $F(X)$ as for scattering from single bilayers provided that $F(0)$, the value of $F(X)$ as X goes to zero, is known for the minus-fluid bilayer (10, 29).

In terms of electron densities, the minus-fluid value of $F(0)$ is given by

$$F(0)/2 = \int_0^{D/2} [\rho^*(x) - \rho_F^*]dx, \qquad (1)$$

where $\rho^*(x)$ is the absolute electron density as a function of distance x along the perpendicular to the bilayer and ρ_F^* is the absolute electron density of the fluid in which the lipid is mixed. The lower limit in Eq. (1) is put in the center of the symmetric bilayer. Because of the subtraction of ρ_F^* the upper limit on the integral need not be specific except that it must include the entire bilayer. Although $F(0)$ is not normally directly measurable, there are three different methods to obtain it from diffraction data on multilamellar arrays. The simplest way uses the relation $\rho^*(D/2) = \rho_F^*$ in the Fourier representation of $\rho(x)$ to yield

$$F(0) = 2\,\Sigma_{h=1}(-1)^{h+1}F(2\pi h/D). \qquad (2)$$

Accuracy of obtaining $F(0)$ using any method is limited by the inability to measure diffraction orders beyond some $h_{\max}$, but a method based on two sets of swelling data (30) and a new method of Worthington (31) as well as a method to be developed in the next paper (28) which require only one set of low angle intensity data, are more accurate than the simple method in Eq. 2.

The main result of this section is derived as follows. Multiple $F(0)/2$ in Eq. 1 by A, the area per lipid molecule along the surface of the bilayer. The integral of A times the first term on the right-hand side of Eq. 1 is given by

$$\int_0^{D/2} \rho^*(x)\, A\, dx = n_L^* + n_F n_F^*, \qquad (3)$$

where n_L^* is the number of electrons in each lipid molecule, n_F^* is the number of electrons in each fluid molecule, and n_F is the number of fluid molecules in the volume of integration which we will write as $AD/2$. (Appropriate averages should be taken on the right-hand side of Eq. 3 for lipid mixtures and for solvent mixtures.) For multilamellar arrays D may be taken to be the lamellar repeat spacing, but it could be different provided only that each lipid molecule is enclosed in the volume $AD/2$. Since this volume contains a lipid molecule and n_F fluid molecules,

$$AD/2 = V_L + n_F V_F, \qquad (4)$$

where V_F is the average volume of the fluid molecules and V_L is the average volume of each lipid molecule. Using Eq. 4 the integral of A times the second term on the right-hand side of Eq. 1 is given by

$$\int_0^{D/2} \rho_F^*\, A\, dx = \rho_F^*[V_L + n_F V_F]. \qquad (5)$$

Using the definition of fluid electron density, $V_F = n_F^*/\rho_F^*$, to eliminate V_F in Eq. 5 and subtracting Eq. 5 from Eq. 3 completes the derivation of the our first relation

$$A\,F(0)/2 = n_L^* - \rho_F^* V_L. \qquad (6)$$

The quantities, n_L^* and ρ_F^*, on the right-hand side of Eq. 6, are known and V_L is obtained from specific volume measurements (14–16).

Relation for the terminal methyl region

It is commonly recognized that an electron density trough in the center of the bilayer represents the terminal methyls on the fatty acid chains. From crystallographic studies it is clear that the methyls have a lower mass density which is manifested as a lower electron density in low angle studies (32). In this section a relation is derived for the average volume V_{CH3} of each terminal methyl in terms of the electron density profiles.

The derivation of the relation begins by considering the integral

$$I^* = \int_0^{x_1} \rho^*(x)\, A\, \mathrm{d}x, \tag{7}$$

where the lower limit is the center of the bilayer and the upper limit, x_1, is any location within the methylene region, i.e., where $\rho^*(x)$ is constant, as indicated in Fig. 1. The integral then gives the number of electrons in the terminal methyls (usually there is one methyl with n^*_{CH3} electrons for each of two fatty acid chains) plus the number of electrons in the n_1 methylenes (each with n^*_{CH2} electrons) that are included in the volume Ax_1. (Note that n_1 is not necessarily the total number of methylenes in the lipid.) Therefore,

$$\begin{aligned} I^* &= 2n^*_{CH3} + n_1 n^*_{CH2} \\ &= 2n^*_{CH3} + n_1 \rho^*_{CH2} V_{CH2}, \end{aligned} \tag{8}$$

where the last equality is obtained using the definition of methylene electron density, $\rho^*_{CH2} = n^*_{CH2}/V_{CH2}$, in terms of numbers of electrons per methylene and the average volume, V_{CH2}, of each methylene. On the other hand, I^* can also be evaluated in terms of the electron density profile, $\rho^*(x)$. In the region of the integration this profile consists of a constant, ρ^*_{CH2}, with a superimposed negative trough. The integrated size of the negative methyl trough will be denoted $2S_M$. (For example, for strip models S_M is given by half the width of the central strip times the difference in electron density of the methylene strip and the central methyl strip. For the model shown in Fig. 1, S_M is half the size of the integrated central Gaussian trough.) Using this notation,

$$\begin{aligned} I^* &= Ax_1\rho^*_{CH2} - AS_M \\ &= (2V_{CH3} + n_1 V_{CH2})\rho^*_{CH2} - AS_M, \end{aligned} \tag{9}$$

where the last equality follows because the volume Ax_1 contains precisely two methyls and n_1 methylenes. To complete the derivation the two expressions for I^* in Eqs. 8 and 9 are now equated and solved for V_{CH3}, yielding

$$V_{CH3} = [n^*_{CH3} + (AS_M/2)]/\rho^*_{CH2}, \tag{10}$$

which is our second relation

Relation for the headgroup region

The same idea that was applied to the methyl region will now be applied to the headgroup region. The integral

$$J^* = \int_{x_H - x_2}^{x_H + x_3} \rho^*(x) A\, \mathrm{d}x \tag{11}$$

is over a volume, $A(x_2 + x_3)$, that completely encloses the headgroup. The lower limit, $x_H - x_2$, is in the constant electron density methylene region and the upper limit, $x_H + x_3$, is in the constant electron density fluid region. Therefore,

$$\begin{aligned} J^* &= n^*_H + n_2 n^*_{CH2} + n_3 n^*_F \\ &= n^*_H + n_2 \rho^*_{CH2} V_{CH2} + n_3 \rho^*_F V_F, \end{aligned} \tag{12}$$

where n^*_H is the number of electrons in the headgroup (which for lecithins we take to include the glycerol backbone and the carbonyls) and n_2 and n_3 are, respectively, the number of methylenes and fluid molecules in the volume of integration, given by

$$A(x_2 + x_3) = V_H + n_2 V_{CH2} + n_3 V_F. \tag{13}$$

There are two important differences between the headgroup region and the methyl region that are reflected in this derivation. As is already seen in Eq. 13 the first is that the region of integration for J^* may contain three kinds of groups instead of just two. The second difference is that the "baseline," upon which is superimposed the headgroup electron density peak, cannot be a simple constant function, as was the case for the methyl region where the baseline was ρ^*_{CH2}, because for the headgroup region the baseline value of electron density is ρ^*_{CH2} at $x_H - x_2$ and it is ρ^*_F at $x_H - x_3$. We will adopt a convention in which the baseline ρ^*_B satisfies the following relation

$$\begin{aligned} &\int_{x_H - x_2}^{x_H + x_3} \rho^*_B(x) A\, \mathrm{d}x \\ &\qquad = [f\rho^*_F + (1 - f)\rho^*_{CH2}]A\,(x_2 + x_3), \end{aligned} \tag{14}$$

where $f = x_3/(x_2 + x_3)$. One example of a baseline that satisfies Eq. 14 is $\rho^*(x) = \rho^*_F(x)$ for $x > x_H$ and $\rho^*(x) = \rho^*_{CH2}(x)$ for $x < x_H$; this consists of a discontinuous step at x_H. Other possibilities for baselines that are not discontinuous at x_H but consist of gradual, monotonic transformations from ρ^*_F at $x_H + x_3$ and to ρ^*_{CH2} at $x_H - x_2$ may easily be constructed (see [28] for example), but no precise functional form is necessary for the derivation. With the characterization of the baseline in Eq. 14, the integrated size, S_H, of the headgroup electron density peak can be quantified as

$$AS_H = \int_{x_H - x_2}^{x_H + x_3} [\rho^*(x) - \rho^*_B(x)]\, A\, \mathrm{d}x. \tag{15}$$

Adding Eqs. 14 and 15 and using Eq. 13 to eliminate $(x_2 + x_3)$ yields

$$\begin{aligned} J^* = AS_H &+ [f\rho^*_F + (1 - f)\rho^*_{CH2}] \\ &\cdot [V_H + n_2 V_{CH2} + n_3 V_F]. \end{aligned} \tag{16}$$

Equating the two expressions for J^*, Eq. 12 and Eq. 16, yields an expression for V_H,

$$V_H = \frac{n^*_H - AS_H + (\rho^*_F - \rho^*_{CH2})[(1 - f)n_3 V_F - f n_2 V_{CH2}]}{f\rho^*_F + (1 - f)\rho^*_{CH2}} \tag{17}$$

Eq. 17 may be combined with Eq. 13 to yield our third and final set of relations,

$$n_2 V_{CH2} = (1 - f)[A(x_2 + x_3) - V_H] - \delta V \quad (18)$$

$$n_3 V_F = f[A(x_2 + x_3) - V_H] + \delta V, \quad (19)$$

where

$$\delta V = \frac{[f\rho_F^* + (1 - f)\rho_{CH2}^*]V_H - n_H^* + AS_H}{\rho_F^* - \rho_{CH2}^*}. \quad (20)$$

DISCUSSION

The first relation, given by Eq. 6, determines the product of the area per molecule, A, and the zeroth order structure factor, $F(0)$, in terms of quantities on the right-hand side of the equation which are known. Therefore, if an absolute electron density profile is obtained, $F(0)$ is easily calculated using Eq. 1 and then Eq. 6 yields the area per molecule, A. In practice, structure factors, including $F(0)$, are often only obtained relative to some unknown scale factor K and so A is also only obtainable to $1/K$ using Eq. 6. As will be discussed in a subsequent paper (28), there are other ways to estimate limits for K so that Eq. 6 is still useful for determining A. The second use of Eq. 6 employs estimates of A obtained from the other studies to estimate the actual value of $F(0)$. Comparing this value of $F(0)$ to the relative value obtained from low angle studies gives the scaling factor K which may then be used to scale $F(X)$ to actual values. From the properly scaled $F(X)$ one can then obtain an absolute electron density profile instead of just the relative ones that are usually presented (1–9).

Our second relation, given by Eq. 10, enables computation of the terminal methyl volume from the area A and from an absolute electron density profile because the latter gives the methylene density, ρ_{CH2}^*, and the size S_M of the methyl trough which are required in the formula. If only the relative electron density is known, then S_M is only known to within the factor K. However, from the first relation, Eq. 6, $1/A$ is also scaled by the same factor K, so the product AS_M remains invariant. The difference, $\rho_{CH2}^* - \rho_F^*$, also scales with K. However, the electron density ρ_{CH2}^* is numerically close to ρ_F^* when the fluid is water, so the variation in ρ_{CH2}^* with K is small. Therefore, the value of V_{CH3} given by Eq. 10 is rather well specified just by a relative electron density profile and is nearly independent of a precise knowledge of K or of A. As will be seen in a subsequent paper (28), the value of V_{CH3} does depend upon the specific functional form that is used to obtain an electron density profile from the data. It may also be noted that in neutron scattering one can obtain more contrast between the methylene region and the fluid region, so the value of V_{CH3} will be more sensitive to the values of K and A; this could provide a useful supplement to x-ray studies.

Our third set of relations for the headgroup region is considerably more complicated than Eq. 10 for the volume of the terminal methyl because the head group divides two regions of different electron densities whereas the terminal methyls divide two methylene regions of the same electron density. Indeed, if ρ_F^* were equal to ρ_{CH2}^*, then Eq. 17 would simplify to an expression similar to Eq. 10. The additional term in the numerator of Eq. 17 involves the quantity, $[(1 - f)n_3V_F - fn_2V_{CH2}]$, which is a weighted difference between the volume of fluid and the volume of methylene in the region of integration around the headgroup. Since neither of these volumes is known, Eq. 17 is of little use in determining V_H, especially since our experience shows that the unknown term makes a modest, but significant, difference to determinations of V_H.

Fortunately, V_H is already known from the relation $V_L = V_H + V_C$, where V_C is the total hydrocarbon volume, which is computed from the sum of the terminal methyl volumes and the methylene volumes. Therefore, our third relation is better thought of as Eqs. 18–20 which provide one Eq. 18 for the amount of water, n_3V_F, and one Eq. 19 for the amount of methylene, n_2V_{CH2}, in the headgroup region defined by the interval $[x_H - x_2, x_H + x_3]$. It has long been clear that the headgroup region should contain fluid molecules which solvate the charged and polar groups on the head. For lipids with inequivalent hydrocarbon chains, such as DPPC (7), it is also clear that the region of constant electron density that contains the methylene in the centers of the chains cannot contain the first few methylenes of the chain that penetrates less far into the bilayer (the two-chain in DPPC) because these methylenes are at the same depth into the bilayer as the carbonyl group on the chain that penetrates further. Therefore, our third relation, expressed by Eqs. 18–20 which require specific volume data and our second relation, connects these structural features in the headgroup region to the electron density profile.

We have found the first two relations to be valuable in the analysis of our low angle x-ray diffraction data from DPPC (28). We have not found the third relation to be useful for x-ray diffraction on the gel phase because the denominator in Eq. 20 is small in this case which magnifies inevitable errors in the numerator to produce large errors in n_2 and n_3 obtained from Eqs. 18 and 19. However, the third relation may also be useful for other phases, other lipids, or neutron diffraction, when the denominator in Eq. 20 is not so small.

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