Definition of Lipid Membrane Structural Parameters from Neutronographic Experiments with the Help of the Strip Function Model

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ABSTRACT Neutron diffraction is an effective method for investigating model and biological membranes. Yet, to obtain accurate structural information it is necessary to use deuterium labels and much time is needed to acquire experimental data as there are a large number of diffraction reflections to register. This paper offers a way to define the hydrophobic boundary position in lipid membranes with high accuracy and for this purpose it is sufficient to take into consideration three structural factors. The method is based on modeling the density of the neutron diffraction amplitude $\rho(x)$ in the direction of the bilayer plane normal by means of a strip function, but it also takes into consideration the fact that the multiplication of the strip function amplitude ρ_i by the step width z_i-z_{i-1} makes the sum of neutron scattering amplitudes of the atoms included in the step region. On the basis of the analysis of a large number of experimental data for different membranes, the effectiveness of this method in the determination of the position of hydrophilic/hydrophobic boundary is demonstrated, including the case of various $\rho(x)$ modifications in the region of polar heads and also the different phase states of membranes. However, it is shown in the present paper that the strip function model is not an adequate instrument for the determination of other structural parameters of membranes.

INTRODUCTION

Neutron diffraction is an effective method for investigating model and biological membrane structure (Worcester, 1983). Primarily it rests on the possible use of deuterium-labeled membrane components, which results in the high accuracy of defining the label position in a membrane (not less than 1 Å) (Büldt et al., 1979; Worcester, 1976). In the opposite case (without the use of deuterium labels), the accuracy of defining the molecule group position is determined by structural resolution (which equals $d/2h_{max}$, where d is the repeat distance of the multilamellar structure and h_{max} is the maximal value of the reflection order) and usually is no better than 4–6 Å (Wornington, 1969). Although the use of deuterium labels is so effective, the process of producing the labeled substance requires special efforts on the chemical synthesis and is expensive (Worcester, 1976). Therefore, it is of great importance to obtain information about a membrane structure from neutronographic experiments without using additional isotopic labels. Previously (King and White, 1986) it was demonstrated on dioleoylphosphatidylcholine (DOPC) membranes that in the case of modeling the density distribution of the neutron scattering amplitude in the normal direction to a lipid bilayer plane with the help of a strip function with high accuracy (which can be compared to the accuracy achieved in the case of using deuterium labels) it is possible to define the hydrophobic/hydrophilic boundary position in lipid bilayers and some other structural parameters of membranes (King and White, 1986; Scherer, 1989).

Another method to determine the structure of the fluid phospholipid bilayer was developed by Wiener and White (1991a,b, 1992). The basic approach is the joint refinement of quasimolecular models with x-ray and neutron diffraction data. This method uses Gaussian functions to describe the distribution of submolecular components. The power of the method has been demonstrated (Wiener and White, 1992), where the structure of the fluid DOPC bilayer was determined. However, 10 quasimolecular fragments were required to obtain the complete structure of dipalmitoylphosphatidylcholine (DPPC) and each piece (Gaussian) is described by three parameters.

The strip function model (King and White, 1986) requires fewer parameters to fix it, which, along with the fact that many problems do not require too much detailed information about bilayers, make the strip function model important.

Nevertheless, for lack of a strict mathematical basis for this procedure it does not automatically follow from King and White (1986) that these methods can be expanded so as to be used with other membranes. Besides, the question remains to be solved of the possibility of using the method (King and White, 1986) to define, for instance, the thickness values of the central membrane region, occupied by CH_3 groups, of the polar bilayer part, and of the water intermembrane layer.

This paper presents the results of investigating the accuracy of determination of membrane structure parameters via the strip function model and its dependence on (1) the number of structural factors used in the process of modeling, (2) the phase state of the membranes, and (3) various structural modifications in the region close to the hydrophilic/hydrophobic boundary. For this purpose we used the

Received for publication 22 February 1995 and in final form 23 June 1995.
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0006-3495/95/10/1424/05 $2.00

experimental structural factors from neutronographic works on 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (1,2-DPPC) (Büldt et al., 1979) and 1,3-DPPC (Büldt and Haas, 1982) membranes in the gel and liquid phases and also from neutronographic experiments performed at the pulsed reactor IBR-2 (Frank and Pacher, 1983) on 1-palmitoyl, 2-oleoyl-phosphatidylcholine, 1,2-DPPC, and dihexadecyl-phosphatidylcholine membranes under other than (Büldt et al., 1979) external conditions and on l-palmitoyl-2-hexadecyl-phosphatidylcholine (PHPC) membranes.

Apart from that, the method (King and White, 1986) was technically modified so that, to determine structural parameters with the help of step function modeling of the scattering amplitude density, three first structure factors were enough.

METHOD

As in King and White (1986) we differentiate between the four characteristic parts of lipid membranes, i.e., the regions containing (1) methyl groups, (2) methylene groups, (3) the polar part of lipid molecule, and (4) water. Let us suppose, according to the Luzzati model (Luzzati, 1968), that water does not penetrate into the polar head region of lipid molecules (a more detailed account for this question will be offered in the future).

The density of the neutron scattering amplitude in the normal direction to a membrane plane $\rho(x)$ can be determined as a step function, the parts of which correspond to the membrane regions enumerated previously (King and White, 1986) (Fig. 1). Let us mark region boundaries by z_1, z_2, z_3, and z_4 and the scattering amplitude densities, corresponding to the steps, by ρ_1, ρ_2, ρ_3, and ρ_4.

Technical modification of the procedure proposed earlier (King and White, 1986) is the following: the multiplication of the step width z_i-z_{i-1} by the corresponding scattering amplitude density ρ_i makes the sum of the scattering amplitudes b_i (of the atoms belonging to this region) divided by the area S of the lipid molecule in the membrane plane. This fact has been used to reduce the number of fitting parameters in the quasimolecular model (Wiener and White, 1992). Let us also suppose that the number of water molecules per a lipid molecule n_w is known (this parameter can be easily defined from gravimetrical measurements).

Thus,

$$\rho_1 z_1 = \sum b_i/S \tag{1}$$

$$\rho_2(z_2 - z_1) = \sum b_i/S \tag{2}$$

$$\rho_3(z_3 - z_2) = \sum b_i/S \tag{3}$$

$$\rho_4(z_4 - z_3) = n_w b_w/S, \tag{4}$$

where b_w is the neutron scattering amplitude of the water molecule. Having in mind that the repeat period is equal to the double value of z_4,

$$2z_4 = d \tag{5}$$

Thus, the introduction of Eqs. 1–4 allowed us to reduce considerably the number of the experimental structure factors sufficient for the full definition of z_i parameters.

The structure factors $F_{strip}(h)$ for the model are defined via the Fourier transformation of $\rho(x)$. Taking into consideration that, usually for lipid membranes $\rho(x)$ is a centrosymmetrical function and substituting $\rho(x)$ by its step model $\rho_{strip}(x)$ under the integral,

$$F_{strip}(h) = \int_{-d/2}^{d/2} \rho_{strip}(x)\cos(2\pi x/d)dx = (d/\pi h)\sum_{i=1}^{h-1}(\rho_i - \rho_{i-1})\sin(2\pi h z_i/d) \tag{6}$$

It follows from Eqs. 1–6 that, to define ρ_i, z_i parameters, it is enough to know the structure factors of three orders of diffraction reflections. It must be noted that the condition of the correspondence of the experimental neutron scattering amplitude density profile,

$$\rho_{obs}(x) = \sum_{h=1}^{h_{max}} F_{obs}(h)\cos(2\pi h x/d) \tag{7}$$

to the step function $\rho_{strip}(x)$ must also be considered; that is, $\rho_{obs}(x)$ has to be close to the step function. The calculations and dependences of $\rho_{obs}(x)$ and $\rho_{strip}(x)$ show that this condition is fulfilled beginning with $h_{max} = 3$. This is an important outcome of using Eqs. 1–4 because the first structure factors are experimentally defined with the highest accuracy, and it usually takes much less time to measure the integral intensities of these reflections than to measure the reflections with $h = 4$ (Worcester, 1983; Wornington, 1969). This result means that, to define the structural parameters of membranes with the help of the step function model, there is no need of measuring high order reflections. ρ_i, z_i parameters are drawn from the comparison of $F_{strip}(h)$ to experimental structure factors $F_{obs}(h)$ via the minimization of the R-factor:

$$R = \left(\sum_h ||F_{strip}(h)| - k|F_{obs}(h)||\right) \Big/ \left(\sum_h k|F_{obs}(h)|\right), \tag{8}$$

$$k = \left[\left(\sum_h |F_{strip}(h)|^2\right) \Big/ \left(\sum_h |F_{obs}(h)|^2\right)\right]^{1/2} \tag{9}$$

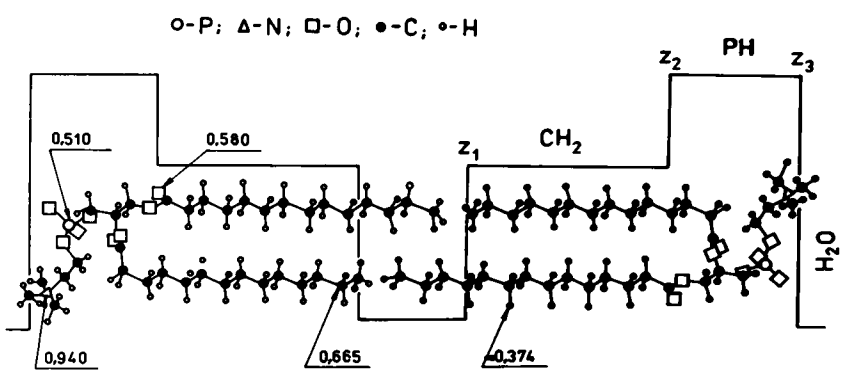

FIGURE 1 A lipid membrane fragment. The neutron scattering amplitude density profiles of a membrane by the step function model in accordance with the conformation of lipid molecules and amplitudes of neutron scattering on nuclei (see the numbers of the corresponding nuclei) are presented. The coordinates (z_i) in the normal direction to a bilayer plane are shown.

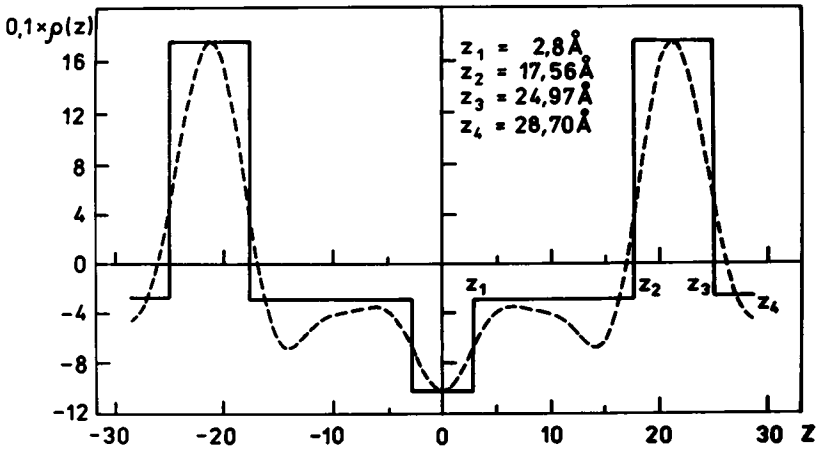

FIGURE 2 Real (dotted line) and model (solid line) neutron scattering amplitude density profiles for DPPC membranes. Experimental structure factors from Zaccai et al. (1979) were used. The coordinate (z) in the normal direction to a bilayer plane is shown in angstroms.

The program STRIPFUN created for a personal computer allows one to calculate, on the basis of Eqs. 1–8, ρ_i, z_i parameters and thus to define the widths of the four molecular zones of lipid membranes, i.e., the water, polar, methylene, and methyl zones. And finally, the *R*-factor is minimized by the variation of molecular zone width values (as in the procedure proposed in King and White, 1986). The program works in the interactive graphic regime and permits one to initiate new starting width values of molecular zones in the process of searching the minimum. Initially x_{max} is being calculated, that is, the position of the maximum of the $\rho_{obs}(x)$ function, which was determined from the experimental structure factors:

$$\rho_{obs}(x) = \sum_h F_{obs}(h)\cos(2\pi hx/d) \qquad (10)$$

The half-width of this maximum Δx is also calculated. The zero-order approximation for the width values of the zones Δz_i is obtained from the following conditions:

$$\Delta z_1 + \Delta z_2 + \Delta z_3 + \Delta z_4 = d/2 \qquad (11)$$

$$\Delta z_1 + \Delta z_2 + \Delta z_3/2 = x_{max} \qquad (12)$$

$$\Delta z_3 = \Delta x \qquad (13)$$

It follows from Eqs. 11–13 that only one of the Δz_i parameters can be chosen independently (the program treats Δz_1 as such).

The limits of changing and the zone width values are then introduced. A physically reasonable maximal value σ of the zone width deviation from the accepted zero approximation is introduced as the initial value. The sizes of the molecular zones are then being defined via the *R*-factor minimization. The value σ is then twice reduced, the minimization procedure is repeated from the last point, and the circle begins again and again until a stable minimum of the *R*-factor is achieved, when the modification of initial conditions does not change the outcome. The σ_k value, at which the minimization process was cut, is in fact the accuracy of defining z_i. In the calculations (with the use of 3–10 structure factors), an accuracy of 0.1–0.5 Å was usually achieved.

Every calculation step is followed by the display demonstration of the *R*-factor value and zone width values as well as $\rho_{obs}(x)$ and $\rho_{strip}(x)$ profiles.

RESULTS AND DISCUSSION

The treatment of experimental data for DOPC membranes from King and White, 1986, with the STRIPFUN program gives the same values as in that paper.

Fig. 2 presents the model and real distributions of the neutron scattering amplitude density on the 1,2-DPPC membrane.

Table 1 shows that the hydrophobic thickness of membranes ($2z_2$), calculated via the strip function model, coincides (with the accuracy of experimental errors) with the corresponding x_0 coordinate values of deuterium labels in the C-2(1) position for 1,2-DPPC, PHPC, and 1,3-DPPC membranes, respectively, determined from DOPC neutron diffraction data.

TABLE 1 Thickness of the hydrophobic part in different membranes under different conditions and its dependence on the number of structure factors (h_{max}) used in the treatment of the data by the strip function model

Membrane	Conditions: Temperature (°C)	Conditions: Relative humidity (%)	Thickness of the hydrophobic layer (in Å) for different numbers of structure factors (h_{max}): 3	4	5	7	8	10	*R*-factor (%)	Thickness of the hydrophobic layer (in Å), determined via deuterium labels	Reference
DOPC	22.5	66 (H_2O)	27.2	28.2					1	27.6	King and White, 1986
1,2-DPPC	20	15 (H_2O)	34.2	35.4	35.2	35.2			5–8	36.8 ± 0.8	Büldt et al., 1979
	70	15 (H_2O)	26.8	27.8	27.6	27.6			1–6	28.8	
1,3-DPPC	20	15 (H_2O)	30.8	34.8	32.2	32.2		31.8	0.1–52	34.0	Büldt and Haas, 1982
	70	60 (H_2O)	26.0	29.8			27.0		0.1–15	26.0 and 28.6	
PHPC	23.4	85 (H_2O)		33.0					0.1		Gordeliy, Anikin, Islamov, Chupin, in preparation
	72	85 (H_2O)		26.8					3		
	72	85 (D_2O)		26					1		

TABLE 2 Width of the polar part (Δz_3) of different membranes under different conditions and its dependence on the number of structure factors (h_{max}) used in the calculations with the strip function model

Membrane	Temperature (°C)	Relative humidity (%)	Width of polar part of lipid bilayer Δz_3 in Å for different h_{max} 3	4	5	7	8	10	Reference
DOPC	22.5	66 (H_2O)	8.8	7.9					King and White, 1986
1,2-DPPC	20	15 (H_2O)	8.7	7.1		7.4			Büldt et al., 1979
	70	15 (H_2O)	9	8.2		7.8			
1,3-DPPC	20	15 (H_2O)	7.8	4.5		6.2		9.1	Büldt and Haas, 1982
	70	60 (H_2O)	6.9		3.4		7.0		
PHPC	23.4	85 (H_2O)	9.4						Gordeliy, Anikin, Islamov, Chupin, in preparation
	72	85 (H_2O)	11.7						
	72	85 (D_2O)	6.3						

The comparison of thickness values from Table 1 for 1,2-DPPC membranes of different hydrations shows that, with increasing hydration, thickness is decreasing. It corresponds to the known fact that an increase of membrane hydration leads to an increase of area per lipid molecule and, accordingly, to an increase of the tilt angle of the chains in relation to the normal of the bilayer plane.

It is also evident that, for $h_{max} \geq 3$, the hydrophobic thickness within the accuracy of the experiment does not depend on the number of structure factors used.

The situation is different with Δzi, the thickness of the other parts of the membrane. The value of Δzi can depend on the number h_{max} used in the treatment of the data. As an example, the calculated values of the widths of the polar part of the lipid bilayer are shown in Table 2.

It should be noted that z_2 does not depend on whether the structure factors of membranes with H_2O or D_2O are used (see Table 1). This is not the case with other structural parameters, for instance, Δz_i. The water layer width Δz_4 is especially sensitive to the isotopic composition of water. In the case of D_2O, we get wrong values of Δz_3 and Δz_4 (Table 2). The second reason for that is that thermal motions in membranes are considerable, which results in smoother boundaries between different parts of the bilayer. It is no wonder, because there is nothing peculiar in the curve of scattering amplitude density at the boundary of these two regions and the extrapolation of these parts of the function $\rho_{obs}(x)$ by the constants is highly probational.

We should also say that, in both the liquid and gel phases, z_2 is defined with high accuracy (see Table 1).

CONCLUSIONS

The proposed procedure of determination of the hydrophobic/hydrophilic boundary of lipid membranes from neutronographic data gives a high accuracy (not less than ±0.5 Å) both in the gel and liquid phases. Three first structure factors are enough to define this boundary, as the z_2 parameter does not depend on the number of the used experimental structure factors and the isotopic composition of water.

Other structural parameters of membranes are more sensitive both to h_{max} and to the isotope composition of water. Large thermal motions in the membrane are one of the reasons for it. To determine these structural parameters, another model of membrane structure can be used (see, for instance, Wiener and White, 1992).

It is evident that both things are determined by the functional behavior of the neutron scattering amplitude density for membranes in the normal direction to the bilayer plane, that is, by $\rho_{obs}(x)$. A drastic change of $\rho_{obs}(x)$ in the carbonyl group region (C═O bond) determines the high accuracy of defining the hydrophobic/hydrophilic boundary in lipid membranes (Fig. 1).

It is probable that, for the same reason in the case of x-ray diffraction on lipid membranes, this procedure can be employed to define the phosphorus position in the membrane polar head. It should be of fundamental importance because the use of both neutronographic and x-ray data would allow one to define with high accuracy the two most important structural parameters: the position of a bilayer surface and the hydrophobic/hydrophilic boundary position without special deuterium labeling.

V. I. Gordeliy is grateful to the Alexander von Humboldt Stiftung for the support of this work. We both thank A. Pole for his help in the preparation of this paper.

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