Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of x-ray and neutron diffraction data

I. Scaling of neutron data and the distributions of double bonds and water

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ABSTRACT We described in two previous papers a method for the joint refinement of the structure of fluid bilayers using neutron and x-ray diffraction data (Wiener, M. C., and S. H. White. 1991*a*, *b*. *Biophys. J.* 59:162–173 and 174–185). An essential part of the method is the appropriate scaling of the diffraction data. Here we describe the scaling of the neutron data and the determination of the transbilayer distribution of double bonds in liquid-crystalline (L_α phase) phospholipid bilayers of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC). The distribution was determined by neutron diffraction of oriented multlayers (66% RH) of DOPC specifically deuterated at the 9- and 10-positions of both acyl chains. The double-bond distribution is described accurately by a pair of Gaussian functions each located at a position Z_{CC} = 7.88 ± 0.09 Å from the bilayer center with 1/e-halfwidths of A_{CC} = 4.29 ± 0.16 Å. Previously, we determined the transbilayer distribution of bromine atoms in a specifically halogenated lipid, 1-oleoyl-2-9,10-dibromostearoyl-*sn*-glycero-3-phosphocholine (OBPC), and showed it to be an isomorphous replacement for DOPC (Wiener, M. C., and S. H. White. 1991*c*. *Biochemistry.* In press). A comparison of the double-bond and bromine profiles indicates that the positions of the centers of the deuterated double bond and the brominated methylene Gaussian distributions are equal within experimental error and that each label undergoes similar average thermal motions with respect to the bilayer normal. The observation that the average position of a label on both acyl chains (the deuterated double bonds) is similar to the average position of a label on the 2-chain alone (the brominated methylenes) indicates that the maximum separation along the bilayer normal between the double bonds of the acyl chains is 1 Å or less. The fully-resolved transbilayer water distribution, previously determined at lower resolution (Jacobs, R. E., and S. H. White. 1989. *Biochemistry.* 28:3421–3437), was obtained from the analysis of neutron diffraction data of DOPC hydrated with a D_2O/H_2O mixture. The water distribution is described accurately by a pair of Gaussian functions each located at a position Z_W = 22.51 ± 0.77 Å from the bilayer center with 1/e-halfwidths of A_W = 4.63 ± 0.48 Å. We present the relative absolute neutron and x-ray structure factors of DOPC at 66% RH that will be used to solve the complete structure of DOPC which will be presented in a later paper of this series.

INTRODUCTION

We are engaged in a detailed investigation of methods for maximizing the information obtainable from lamellar diffraction of lipid bilayers. Two earlier papers established the theoretical foundations of our methods of "liquid-crystallography." The first (Wiener and White, 1991*a*) examined critically the issue of resolution in membrane diffraction and the role of appropriate models in structural refinement. Quasimolecular models that represent the liquid-crystalline bilayer in terms of a series of multiatomic Gaussian distributions were shown to provide a physically meaningful real-space representation of the distribution of matter in the bilayer. The second (Wiener and White, 1991*b*) described the composition-space refinement method which enables the simultaneous consideration of neutron and x-ray diffraction data in bilayer structure determination. A requirement of the joint-refinement procedure is that the neutron and x-ray structure factors be on the appropriate absolute scales (King et al., 1985; Jacobs and White, 1989; Wiener and White, 1991*b*). The analysis of structural isomorphs permits such determinations (Wiener and White, 1991*c*). In this paper, we present the properly scaled neutron and x-ray structure factors of liquid-crystalline (L_α phase) DOPC that will be used for the determination of the complete structure of DOPC described in a subsequent paper. In addition, we describe the transbilayer distribution of the double bonds in liquid-crystalline (L_α phase) phospholipid bilayers of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) determined by neutron diffraction of oriented multilayers of DOPC at 66% RH specifically deuterated at the 9- and 10-positions of both acyl chains. This is, to our knowledge, the first direct determination of the double-bond distribution in a liquid-crystalline bilayer.

The double-bond distribution is described accurately by a pair of Gaussian functions located a distance Z_{CC} = 7.88 ± 0.09 Å from the bilayer center with 1/e-halfwidths of A_{CC} = 4.29 ± 0.16 Å. Previously, we determined the

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transbilayer distribution of the bromine atoms in a specifically halogenated lipid, 1-oleoyl-2-9,10-dibromostearoyl-*sn*-glycero-3-phosphocholine (OBPC), that was shown to be an isomorphous replacement for DOPC (Wiener and White, 1991*c*). A comparison of the double bond and bromine profiles sets an upper bound of 1 Å on the separation along the bilayer normal between the double-bond positions of the two acyl chains. We also present the transbilayer water distribution obtained from neutron diffraction of DOPC hydrated with a D_2O/H_2O mixture. The water distribution is described accurately by a pair of Gaussian functions located a distance $Z_W = 22.51 \pm 0.77$ Å from the bilayer center with 1/e-halfwidths of $A_W = 4.63 \pm 0.48$ Å.

We emphasize in this paper the systematic scaling of neutron diffraction data. Whereas the basic principles of scaling neutron diffraction data are well known (Zaccai et al., 1975; Worcester and Franks, 1976; Büldt et al., 1979; King et al., 1985; Jacobs and White, 1989), there are subtleties to the appropriate treatment of data that have only recently become apparent during our development of the joint refinement method. For this reason we found it imperative to reanalyze all of the relevant neutron diffraction data before the complete joint refinement structure determination of DOPC which will be presented in a later paper of this series. Although some of the unscaled neutron diffraction data in this paper have been previously published, a complete and rigorous scaling has heretofore not been performed. Specifically, eight orders of neutron diffraction data of DOPC on an arbitrary scale were presented by King and White (1986) and the first four orders (h = 1–4) of that data set were reprinted by Jacobs and White (1989) in their study of peptide-bilayer mixtures. Jacobs and White (1989) presented four orders, also on an arbitrary scale, of DOPC hydrated with 20 mol% D_2O and used these data to obtain a transbilayer water distribution. In this paper, we present previously unpublished data of DOPC specifically deuterated at the double bonds and use these data to obtain the first, to our knowledge, direct determination of a transbilayer double-bond distribution in a liquid-crystalline bilayer. Also, we present, for the first time, higher order (h = 5–7) data of DOPC hydrated with 20 mol% D_2O and use the seven-order data to obtain a more robust and fully-resolved water distribution than that presented previously by Jacobs and White (1989). Lastly, we have included in tabular form, the relative absolute x-ray structure factors determined from x-ray diffraction of a halogenated structural isomorph of DOPC (Wiener and White, 1991*c*). Because the relative absolute neutron and x-ray structure factors of DOPC are utilized as the input of the final joint refinement structure determination, the tabulation of both data sets is a convenient prelude for subsequent papers of this series.

MATERIALS AND METHODS

Materials

1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was obtained from Avanti Polar Lipids (Birmingham, AL) and used without further purification. DOPC specifically deuterated (95%) at the double-bond positions (C-9 and C-10) of both acyl chains was obtained from Cambridge Isotope Laboratories (Woburn, MA) and purified by preparative thin layer chromatography. Analytical thin layer chromatography of both labeled and unlabeled DOPC yielded single spots using 13:7:1 $CHCl_3$:MeOH:NH_4OH as a solvent.

Neutron diffraction

Neutron diffraction measurements were made at Brookhaven National Laboratory (Upton, New York) using the High Flux Beam Reactor. The experimental methods (White et al, 1981; King et al, 1985; King and White, 1986; Jacobs and White, 1989) have been previously described in detail.

Scaling experimental data

White and co-workers (King et al., 1985; White and Jacobs, 1989; Wiener and White, 1991*b*, *c*) developed a convenient "relative absolute" scale that permits the detailed examination and modeling of diffraction data without explicit knowledge of the lipid cross-sectional area S. This seemingly contradictory term was adopted because there are three parts of the scaling problem. The first is to establish the correct mean level of the transbilayer profile with respect to the absolute zero of scattering density. The second is to establish the proper relative amplitude of the fluctuation of the profile with respect to this mean level on a per unit cell basis. These two steps place the profile on an absolute scale with respect to the unit cell contents. This scale is sufficient for determining the composition-space structure of the bilayer (Wiener and White, 1991*b*). However, the placement of the profile on the absolute per volume scale requires knowledge of the area per lipid (S). Therefore, another scaling is required in this third step and we thus refer to the scale at the end of step two as the "relative" absolute scale. A complete development of the relative absolute scale is given by Jacobs and White (1989; see in particular Eqs. 2–4). Briefly, the relative absolute density profile $\rho^*(z)$ is given by (Jacobs and White, 1989; Wiener and White, 1991*b*, *c*)

$$\rho^*(z) = \rho_o^* + (2/dk) \sum_{h=1}^{h_{max}} f(h) \cos(2\pi hz/d). \quad (1)$$

The first term on the right-hand-side of the equation, ρ_o^*, is the average density per unit length of the bilayer. The second term describes the fluctuations in scattering length about this average across the bilayer. Experiments yield sets of structure factors $f(h)$ that are related to the relative absolute structure factors $F^*(h)$ by instrumental scaling constants k (King et al., 1985; Jacobs and White, 1989; Wiener and White, 1991*b*, *c*)

$$f(h) = kF^*(h). \quad (2)$$

The scale factor k has no physical connection to the bilayer structure but is related solely to the experimental set up. In neutron diffraction, a molecule (A) and a specifically deuterated variant (B) form an isomorphous replacement pair. Given two sets of measured structure factors $f_A(h)$ and $f_B(h)$, the scale factors k_A and k_B can be determined by relating the structures of A and B to absolute scattering space through a comparison of their relative absolute Fourier density profiles. At a point $z = z_1$ where the two profiles are identical, $\rho_A^*(z) = \rho_B^*(z)$ and

$$(2/dk_A) \sum_{h=1}^{h_{max}} f_A(h) \cos(2\pi h z_1/d) - (2/dk_B) \sum_{h=1}^{h_{max}} f_B(h) \cos(2\pi h z_1/d) = \rho_{oB}^* - \rho_{oA}^* = \Delta\rho^*. \quad (3)$$

If the difference $\Delta\rho^*$ in average densities of the two samples is known, two "match-points" z_1 and z_2 are sufficient to determine k_A and k_B which yields the relative absolute structure factors $F_A^*(h)$ and $F_B^*(h)$. In this paper, two isomorphous replacement pairs used in the neutron diffraction experiments are examined: (*a*) DOPC and DOPC with deuterated double-bonds, (*b*) DOPC hydrated with pure H_2O and DOPC hydrated with a mixture of 20 mol% D_2O and 80 mol% H_2O. Scaling of the x-ray data was carried out by the analysis of a series of mixtures of DOPC and a specifically brominated isomorph, 1-oleoyl-2-9,10-dibromostearoyl-*sn*-glycero-3-phosphocholine (OBPC), and is described by Wiener and White (1991*c*).

Determining the distribution of a label

The distributions of specifically labeled regions of bilayers can often be described by Gaussian distributions (Büldt et al., 1978; Franks et al., 1978; Büldt et al., 1979; Zaccai et al., 1979; Jacobs and White, 1989; Wiener and White, 1991*c*) which reflect the thermal disorder of liquid-crystalline bilayers (Wiener and White, 1991*a*). After the two data sets of an isomorphous replacement pair (A and B) are scaled, relative absolute difference structure factors $\Delta F^*(h)$ are calculated

$$\Delta F^*(h) = f_B^*(h)/k_B - f_A^*(h)/k_A. \quad (4)$$

For a Gaussian distribution with center Z, 1/e-halfwidth A, and area b (the difference in scattering length between labeled and unlabeled molecules), the difference structure factors are given analytically (Franks et al., 1978; Wiener and White, 1991*c*) by

$$\Delta F^*(h) = 2b \exp(-(\pi A h/d)^2) \cos(2\pi h z), \quad (5)$$

and the parameters of the distribution are obtained by fitting Eq. 5 to $\Delta F^*(h)$ with a minimization of the crystallographic R factor.

RESULTS AND DISCUSSION

The DOPC neutron diffraction data were scaled in combination with data from DOPC deuterated at the double bonds. We chose to scale the DOPC data in this manner because the difference in average density ($\Delta\rho^*$ in Eq. 3) between the deuterated double-bond analogue and DOPC ($\Delta\rho^* = 0.1611 \cdot 10^{-4}$) is larger than the difference in average density between DOPC hydrated with 20 mol% D_2O/80 mol% H_2O and DOPC hydrated with 100 mol% H_2O ($\Delta\rho^* = 0.0906 \cdot 10^{-4}$); values of ρ_0^* are listed in Table 1. Four orders of data were recorded for the deuterated analog. As will be seen, the experimental difference profile agrees very well with the four-order Fourier reconstruction of the Gaussian function that describes the double-bond distribution. Therefore, the

TABLE 1 **Relative absolute neutron and x-ray structure factors $F^*(h)$ for the observed orders of diffraction determined for oriented multilayers of DOPC bilayers at 66% RH**

	$F^*(1)$	$F^*(2)$	$F^*(3)$	$F^*(4)$	$F^*(5)$	$F^*(6)$	$F^*(7)$	$F^*(8)$
Neutron diffraction								
(100 mol% H_2O)[§]; $\rho_0^* = 0.1234 \cdot 10^{-4}$								
	−8.00 ± 0.44	−4.51 ± 0.24	4.81 ± 0.25	−5.18 ± 0.29	−0.59 ± 0.08	0.84 ± 0.11	0.0 ± 0.08	−0.94 ± 0.14
(20 mol% D_2O/80 mol% H_2O)[‖]; $\rho_0^* = 0.2140 \cdot 10^{-4}$								
	−11.97 ± 0.65	−1.77 ± 0.10	3.38 ± 0.19	−4.74 ± 0.26	−0.67 ± 0.09	0.83 ± 0.11	0.0 ± 0.09	n.o.
Deuteration at double bonds (100 mol% H_2O); $\rho_0^* = 0.2845 \cdot 10^{-4}$								
	−4.18 ± 0.19	−7.03 ± 0.32	0.87 ± 0.10	−6.74 ± 0.30	n.o.	n.o.	n.o.	n.o.
X—Ray diffraction[¶]; $\rho_0^* = 5.6046 \cdot 10^{-4}$								
	−43.95 ± 0.88	−0.52 ± 0.52 −0.74**	5.15 ± 0.80	−11.97 ± 1.29	3.38 ± 0.32	−2.47 ± 0.88	2.03 ± 0.65	−2.24 ± 0.49

The D_2O/H_2O compositions of the saturated $NaNO_2$ solutions used to control the relative humidity of the multilayers in the neutron experiments are indicated. The d-spacing of DOPC at 66% RH is 49.1 ± 0.3Å[‡]. ρ_0^*, on the relative absolute scale, is dimensionless (i.e., scattering length per length).

n.o. Not observed.

[‡] Jacobs and White (1989).

[§] The experimental structure factors $f(h)$ that were used in the scaling calculation were reported by King and White (1986).

[‖] Four orders (h = 1–4) of the experimental structure factors $f(h)$ that were used in the scaling calculation are given by Jacobs and White (1989).

[¶] These data are presented (but not tabulated) in Fig. 3 of Wiener and White (1991c); a complete description of the x-ray diffraction experimental methods is also presented in that paper.

**The calculated error for the second order is ±0.74, which implies that $F^*(2)$ could actually be positive (−0.52 + 0.74). Because the independent phasing of the structure factors determined the phase of the second order to be −1 (Wiener and White, 1991c), the quoted error is reduced to indicate the exclusion of positive values of $F^*(2)$.

errors in scaling and determination of the double-bond distribution that arise from analyzing four instead of h_{max} (eight) structure factors are negligible. Two match-points, $z_1 = d/2 = 24.55$ Å and $z_2 = 18$ Å, were used to determine the two instrumental scale factors (Eq. 3) and the resultant relative absolute profiles, calculated from Eq. 1, are shown in Fig. 1 *A*. A Gaussian function was fit to the difference structure factors $\Delta F^*(h)$ in reciprocal space (Eq. 5) and is shown in Fig. 2 *A*. The double-bond distribution is accurately represented as a pair of Gaussians that are each $Z_{CC} = 7.88 \pm 0.09$ Å from the bilayer center with 1/e-halfwidths of $A_{CC} = 4.29 \pm 0.16$ Å. The relative absolute DOPC data were then used to scale the remaining data set, seven orders recorded from DOPC hydrated with 20 mol% D_2O/80 mol% H_2O. In this case, only one match-point ($z = 0$ Å) is required because one of the data sets is already on the relative absolute scale. If these two data sets, DOPC hydrated with 20 mol% D_2O/80 mol% H_2O and DOPC hydrated with 100 mol% H_2O, are used first to scale the data, the DOPC scale factor (0.121 ± 0.006) is consistent with the value obtained from using the DOPC and deuterated double-bond data sets (0.118 ± 0.004). The relative absolute profiles are shown in Fig. 1 *B* and the best Gaussian fit of

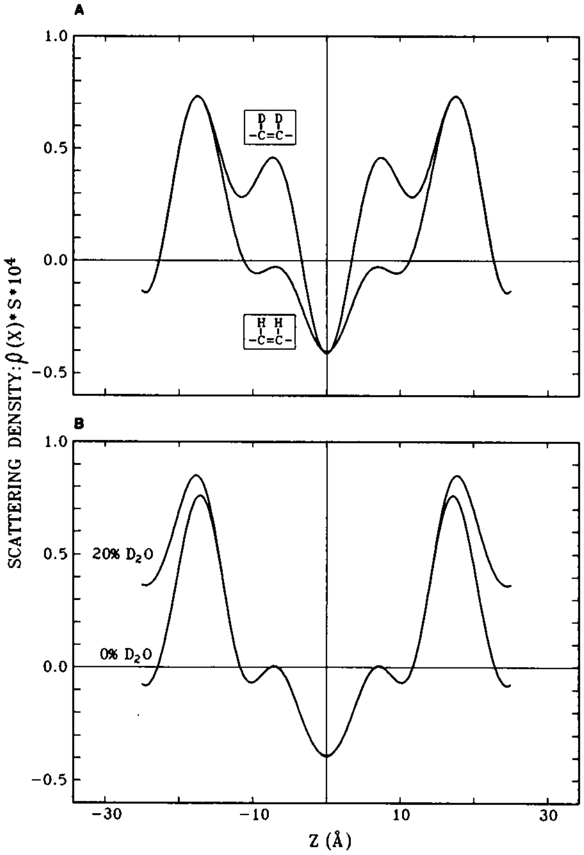

FIGURE 1 Relative absolute density profiles obtained from neutron diffraction of DOPC and several structural isomorphs. The scale factors that place the experimental structure factors on the relative absolute scale were determined from Eq. 3. (*A*) Four-order profiles of DOPC and DOPC deuterated at the double bonds of both acyl chains. The profiles were matched at $z_1 = d/2 = 24.55$ Å and $z_2 = 18$ Å. (*B*) Seven-order profiles of DOPC hydrated with 100 mol% H_2O and DOPC hydrated with a mixture of 20 mol% D_2O and 80 mol% H_2O. The profiles were matched at the single point $z_1 = 0$ (the scale factor for DOPC hydrated with 100 ml% H_2O was already determined from *A*).

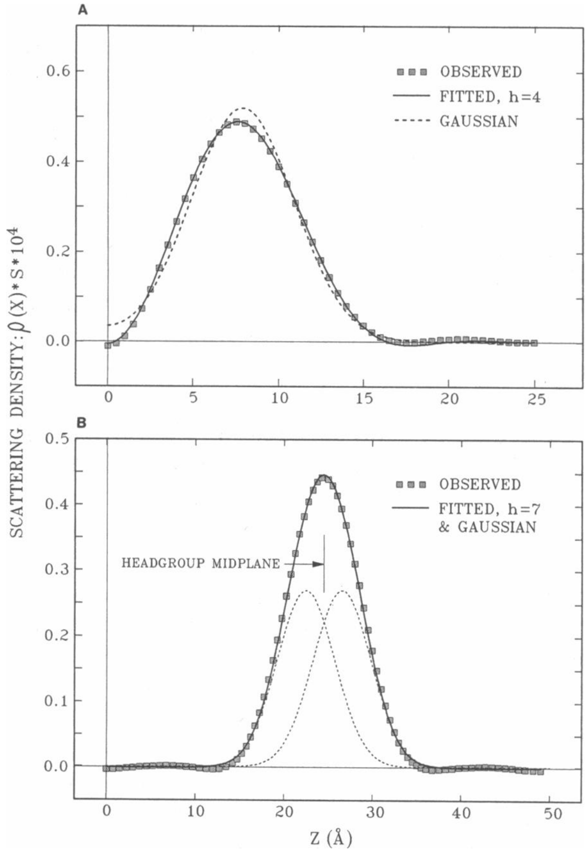

FIGURE 2 The double bond and water distributions determined from the relative absolute difference structure factors of the two isomorphous pairs. The difference structure factors were fit to Eq. 5 with minimization of the crystallographic R factor. (*A*) The double-bond difference-structure profile and the Gaussian distribution ($Z_{CC} = 7.88 \pm 0.09$ Å and $A_{CC} = 4.29 \pm 0.16$ Å) fit to it. The difference between the actual Gaussian and the four-order reconstruction shows that $h = 4$ orders is insufficient to provide a fully-resolved image of a Gaussian of this width. However, the four-order reconstruction of the Gaussian is a good fit to the experimental data. (*B*) The water difference-structure profile and the Gaussian distribution ($Z_W = 22.51 \pm 0.77$ Å and $A_W = 4.63 \pm 0.48$ Å) fit to it. The dotted lines are the water distributions of two apposed bilayers and the larger envelope is their sum. In contrast to the double-bond distribution, the seven-order reconstruction of the Gaussian is virtually identical to the Gaussian itself.

the water distribution is shown in Fig. 2 *B*. The water distribution is accurately represented as a pair of Gaussians, each $Z_W = 22.51 \pm 0.77$ Å from the bilayer center with 1/e-halfwidths of $A_W = 4.63 \pm 0.48$ Å. This distribution is similar in appearance to water profiles obtained in previous neutron diffraction studies (Zaccai et al., 1975; Worcester and Franks, 1976; Jacobs and White, 1989).

The uncertainties in the Gaussian parameters were determined by a Monte Carlo sampling procedure (Press et al., 1989; Wiener and White, 1991*b, c*). Each value of a structure factor and its associated error are used to define a normal distribution. Sets of mock data are generated by random sampling from these normal distributions and the scaling and Gaussian fitting calculations are repeated with each new "noisy" data set as input. The results of $N = 1,000$ iterations were averaged and the standard deviations of Z_{CC}, A_{CC}, Z_W and A_W from these simulations serve as estimates of the parameter uncertainties. We note that in earlier papers (King and White, 1986; Jacobs and White, 1989), the water distribution in each half of the bilayer was fit by two Gaussian distributions. With the present more refined modeling procedure, not utilized previously, the two-Gaussian model was not as robust as a one-Gaussian model in the sense that the envelope of the total water distribution could be fit by a large number of Gaussian pairs. Also, the reduced R factors (i.e., the crystallographic R factors divided by the degrees of freedom) are virtually equal for the one- and two-Gaussian fits. We use the more parsimonious model in this paper and subsequent publications but it should be emphasized that the number of Gaussians representing the water distribution does not affect the earlier conclusions of Jacobs and White (1989).

Crystal structures of phospholipids (Hitchcock et al., 1974; Pearson and Pascher, 1979; Hauser et al., 1981) and neutron diffraction of specifically-deuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) in the gel phase (Büldt et al., 1978, 1979; Zaccai et al., 1979; Büldt and Seelig, 1980) indicate that the two all-*trans* acyl chains are positionally inequivalent with respect to the bilayer normal. The glycerol moiety is approximately normal to the bilayer surface. Therefore, the 1-chain penetrates more deeply into the bilayer interior because the 2-chain proceeds approximately parallel to the membrane surface from the glycerol backbone and then bends inward towards the hydrocarbon center. ^{2}H-NMR studies of specifically-deuterated liquid-crystalline L_α-phase phospholipid bilayers demonstrate that the two acyl chains are dynamically inequivalent on the NMR time scale (see reviews by Seelig, 1977; and Browning, 1981). In particular, ^{2}H-NMR of DOPC deuterated at the 9,10 positions of both double bonds yielded four distinct quadrupole splittings, one for each deuteron (Jacobs and White, 1984). In this context of chain inequivalence, it is of interest to compare our results on the double-bond distribution obtained from neutron diffraction (where both chains were labeled) with our previous determination of the transbilayer bromine distribution of 1-oleoyl-2,9,10-dibromostearoyl-*sn*-glycero-3-phosphocholine (OBPC) (Wiener and White, 1991*c*).

Previously, we demonstrated that OBPC is a satisfactory isomorphous replacement for DOPC and the analysis of a series of OBPC/DOPC mixtures was used to place the DOPC x-ray data on the relative absolute scale and determine the bromine distribution. The bromine atoms at the 9,10-positions are accurately described by a pair of Gaussian functions each $Z_{Br} = 7.97 \pm 0.27$ Å from the center of the bilayer with 1/e-halfwidths of $A_{Br} = 4.96 \pm 0.62$ Å. The double bond and bromine distributions are compared in Fig. 3. The position of the double bond, $Z_{CC} = 7.88 \pm 0.09$ Å, agrees within experimental error with the bromine position Z_{Br}. The 1/e-halfwidth of the double-bond, $A_{CC} = 4.29 \pm 0.16$ Å, is approximately 0.7 Å smaller than the bromines' halfwidth. A simple calculation (Wiener and White,

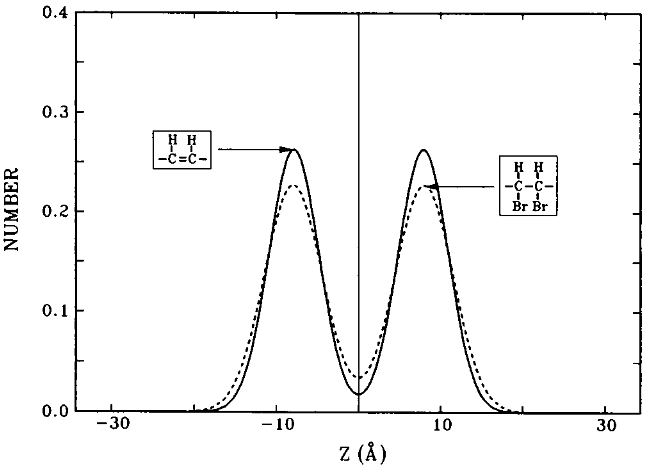

FIGURE 3 A comparison of the double-bond distribution obtained from specifically deuterated DOPC and the transbilayer bromine distribution of 1-oleoyl-2-9,10-dibromostearoyl-*sn*-glycero-3-phosphocholine (OBPC), a specifically brominated isomorph of DOPC (Wiener and White, 1991*c*). The centers of the two distributions, $Z_{CC} = 7.88 \pm 0.09$ Å and $Z_{Br} = 7.97 \pm 0.27$ Å, are equal within experimental error. The differences in their 1/e-halfwidths, $A_{CC} = 4.29 \pm 0.16$ Å and $A_{Br} = 4.96 \pm 0.62$ Å, can be ascribed to the difference in size between deuterium and bromine atoms. The underlying ranges of thermal motion of each label are similar (see text).

1991*c*) demonstrates that this variation is most likely due primarily to the difference in size between deuterons and the bulkier bromine atoms used as labels in x-ray diffraction. The distribution of a localized label across the bilayer can be represented as the convolution of two distributions: the hard sphere or van der Waals stationary distribution of the label and an envelope of thermal motion. If each of these distributions, hard sphere and thermal, is represented by a Gaussian of 1/e-halfwidth A_H and A_T, respectively, their convolution product is another Gaussian of 1/e-halfwidth $(A_H^2 + A_T^2)^{1/2}$ (Hosemann and Bagchi, 1962). For the bromine atoms, the hard-sphere halfwidth A_H was estimated to be ~3.0 Å (Wiener and White, 1991*c*), which yields a thermal envelope with a 1/e-halfwidth A_T = 3.9 ± 0.5 Å. The hard-sphere radius A_H of the deuterated double bond, estimated from values of covalent bond lengths and van der Waals radii (Pauling, 1960), is ~2.2 Å. This value yields a thermal envelope with a 1/e-halfwidth A_T = 3.7 ± 0.5 Å, which provides an indication of the high degree of thermal disorder in liquid-crystalline bilayers (Wiener and White, 1991*a*). The agreement, within experimental error, between the thermal envelopes of the deuterated double bonds and the brominated single bonds at the 9,10 positions implies that the average thermal motions of the two labels projected onto the bilayer normal are similar. Because the double bonds are dynamically inequivalent on the NMR time-scale (vide supra), this would suggest that the complex three-dimensional thermal motions of each double bond have similar long-time averages projected onto the bilayer normal.

We explored the ability of the analyses of the double bond and bromine distributions to discern spatial chain inequivalence along the bilayer normal. First, the neutron diffraction difference structure factors were fit by a two-Gaussian distribution instead of the single Gaussian used earlier. The two-Gaussian fit was constrained by forcing the two distributions, one for each double bond, to have equal 1/e-halfwidths. Interestingly, the two-Gaussian fits had R factors that were virtually identical to those of the original one-Gaussian fit, i.e., the extra parameter in the two-Gaussian model did not lead to any improvement in the fit. By Occam's razor, if both the one- and two-Gaussian structural models provide adequate fits then there is no intrinsic reason to favor the two-Gaussian model on the basis of the neutron diffraction difference structure factors alone. However, the failure of the four-order neutron data alone to resolve two separate double-bond distributions does not rule out completely the possibility that two separate distributions at different average positions is the more accurate representation of the double-bonds of DOPC at 66% RH. One could, in principle, postulate alternatively that the position of the bromine atoms of the 2-chain located by x-ray diffraction (7.97 ± 0.27 Å) is indeed the position of the double-bond of the 2-chain of DOPC and that the double-bond of each chain is equidistant from the center of the distribution of both chains' double-bonds, Z_{CC} = 7.88 ± 0.09 Å, that was determined from neutron diffraction. With the conventional assumption that the 1-chain penetrates more deeply into the bilayer interior, we can estimate a limiting value of Z_{CC} [2-chain] ≈ 7.97 + 0.27 = 8.24 Å. The largest displacement from the position Z_{CC} determined from neutron diffraction is then given by (8.24–[7.88–0.09]) = 0.45 Å. The double bond of the 1-chain would then be estimated to be centered at a position ([7.88–0.09]–0.45) = 7.34 Å so that the maximum separation of the two double bonds that is consistent with both x-ray and neutron data is ~1 Å.

We conclude that the separation of the two chains at their respective double-bond positions projected along the bilayer normal in L_α-phase DOPC at 66% RH, which corresponds to 5.4 waters per lipid (White et al., 1987), is ≈1 Å or less. For comparison, the crystal structure of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) (Pearson and Pascher, 1979) has an offset between the acyl chains of 4.1–4.3 Å with respect to the chain axis. In gel-phase DPPC at low hydration, 2.6 waters per lipid, the separation between the C-2 carbon positions along the bilayer normal is ~1.8 Å (Büldt et al., 1978; Zaccai et al., 1979); because the acyl chains in DPPC at this hydration are tilted with respect to the bilayer surface by 17° (Tardieu et al., 1973), the separation with respect to the chain axis is slightly higher (1.9 Å). In going from the most highly-ordered all-*trans* crystalline state to the gel phase, which is still an all-*trans* state, the size of the offset decreased by a factor of two. It is reasonable that in the chain-melted L_α phase, where the hydrocarbon thickness is ~25% smaller than in the gel phase (Büldt et al., 1978; Zaccai et al., 1979), this separation will decrease further in accord with our observation. A more complete answer to the question of positional chain inequivalence awaits neutron diffraction of a series of liquid crystalline bilayers that are appropriately deuterated on individual acyl chains. The close agreement between the distributions of the deuterated double bonds of DOPC and the brominated single-bond of OBPC (Fig. 3) reinforces the idea (East and Lee, 1982) that substituting bulky bromine atoms for the hydrogens on adjacent methylenes produces a feature on the acyl chain that mimics a double bond (at least under the conditions of these experiments).

The relative absolute neutron $[F_N^*(h)]$ and x-ray $[F_X^*(h)]$ structure factors of DOPC at 66% RH are given in Table 1. The relative absolute structure factors of DOPC with deuterated double bonds and DOPC hydrated with 20 mol% D_2O/80 mol% H_2O are also provided. $F^*_N(h)$ and $F^*_X(h)$ will be used to determine the complete structure of DOPC, via the composition-space joint refinement method (Wiener and White, 1991*b*), that will be presented in a later paper. In order to determine the accuracy of structures obtained from the joint-refinement procedure, it is essential to characterize the uncertainty in the relative absolute structure factors. Two factors contribute to the total errors given in Table 1. First, the precision of the experimental structure factors $f(h)$ places a bound on the ultimate accuracy of the relative absolute structure factors $F^*(h)$ that are based upon them. The experimental errors in the neutron structure factors are determined by the statistics of a small number of experiments (Jacobs and White, 1989). The experimental errors in the x-ray structure factors are based upon the results of a multiple linear regression analysis of a large number of experiments over a range of OBPC/DOPC concentrations (Wiener and White, 1991*c*). This error can be expressed as an experimental "self-R," analogous to the crystallographic R factor, that is the sum over $h_{\max}$ observed diffraction orders of the absolute values of the experimental errors divided by the sum of the absolute values of the experimental structure factors $f(h)$ (Wiener and White, 1991*a*). The self-R of the experimental neutron and x-ray structure factors of DOPC are 0.031 and 0.076, respectively. Second, calculation of the instrumental scale factors that place the data on the relative absolute scale requires the selection of several match-points (Eq. 3), i.e., points where the densities of the labeled and unlabeled molecules are set equal to each other. Inspection of Fig. 1 (and Fig. 4 of Wiener and White, 1991*c*) reveals that each of the pairs of molecules are satisfactory structural isomorphs, i.e., the two profiles are very similar except in the region of the label. We examined the variation in the instrumental scale factors as a function of the positions of the match points. The variation in the neutron and x-ray instrumental scale factors for DOPC are 3.4 and 0.8%, respectively. The two errors, experimental precision and scale factor variation, were combined to arrive at the uncertainties of Table 1. The final relative absolute self-R factors of the neutron and x-ray data sets are 0.065 and 0.084, respectively. Fig. 4 depicts the relative absolute neutron and x-ray profiles of DOPC at 66% RH. The variation in each profile is the standard deviation of a large number (1,000) of profiles that were generated by Monte Carlo sampling (vide supra) of the structure factors in Table 1 and averaged in real-space.

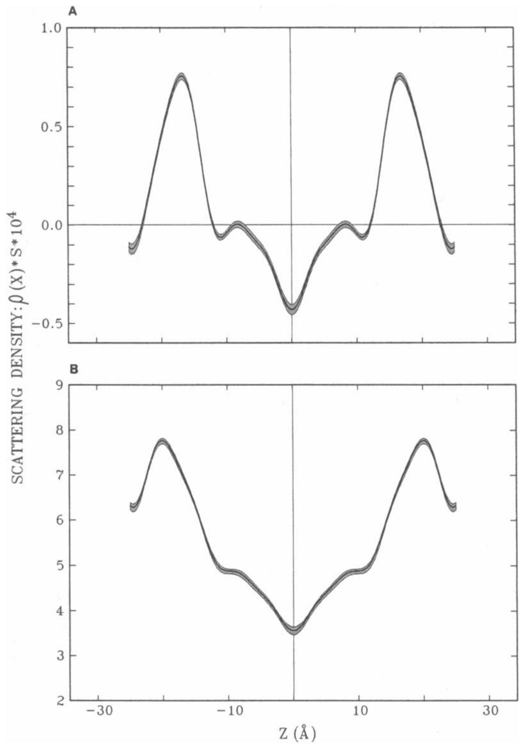

FIGURE 4 Neutron and x-ray scattering relative absolute density profiles for DOPC at 66% RH. The variation in each profile is the standard deviation of a large number (1,000) of Fourier profiles that were generated by Monte Carlo sampling (see text) of the structure factors in Table 1 and averaged in real space. (*A*) Relative absolute neutron scattering density profile. (*B*) Relative absolute x-ray scattering density profile.

There are several clear and obvious differences between the relative absolute neutron and x-ray scattering length density profiles shown in Fig. 4. The differences between neutron and x-ray scattering lengths in the context of biological membranes have been noted by others (e.g., Schoenborn, 1975; Worcester, 1976; Franks and Lieb, 1979, 1981). First, the negative neutron scattering length of hydrogen (Sears, 1986) has the effect of placing the average neutron scattering density of the bilayer ($0.1234 \cdot 10^{-4}$) close to the absolute zero of scattering density so that the bilayer profile can be viewed as a series of positive and negative fluctuations about zero density. In contrast, the average x-ray scattering length density ($5.6046 \cdot 10^{-4}$) is much greater than the absolute zero. Second, neutrons scatter most strongly from regions of the bilayer with the smallest concentration of hydrogens, i.e., the carbonyls make the primary contribution to the headgroup peak at ≈17 Å in the neutron profile. Also, the double bonds are a significant scattering feature and the peak in the neutron density profile at 8.4 Å is within 0.5 Å of the double-bond position determined by specific labeling (7.88 ± 0.09 Å). X-rays scatter most strongly from electron-dense regions so that the headgroup feature at ≈20 Å arises primarily from the phosphate moiety. Third, the magnitudes of the density fluctuations in the neutron density profile are 3–4 times smaller than the fluctuations in the x-ray density profile. These differences reflect the fact that each experimental technique "sees" a representation of the bilayer in a different scattering space. The images obtained from each experiment are independent so a structure determined from the joint analysis of both of these images is more detailed than a structure based upon a single data set. On the diffraction time scale, a single average distribution of matter in the bilayer gives rise to each of these independently-recorded images, and this basic realization underlies our joint-refinement method (Wiener and White, 1991*b*).

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