

## Hydration effect on the structure of dioleoylphosphatidylcholine bilayers

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*In situ* energy dispersive x-ray diffraction was used to study the effect of hydration on the structure of dioleoylphosphatidylcholine (DOPC) bilayers in the biologically relevant liquid-crystalline phase. A broad range of relative humidity (RH) was investigated ( $0.45 < \text{RH} < 1$ ) and two hydration regimes were found. From  $\text{RH}=0.45$  up to  $\text{RH}\sim 0.94$ , the structure of DOPC bilayers was found to change slightly. At  $\text{RH}\sim 0.94$ , a distinctive structural change was observed. From  $\text{RH}\sim 0.94$  up to full hydration, a strong increase in the head group size, coupled with a marked decrease in the hydrophobic core thickness, was observed. Possible molecular mechanisms underlying experimental observations are discussed. © 2007 American Institute of Physics. [DOI: 10.1063/1.2734470]

The interaction of water molecules with the lipid polar head groups is one of the fundamental aspects of biological membrane assembly.<sup>1</sup> Experiments with phospholipid model membranes have shown that the degree of head group hydration affects both the membrane structure and dynamics.<sup>2,3</sup> The molecular packing<sup>4</sup> and the interaction between bilayers<sup>5</sup> depend on the amount of water associated with the lipids. The chain melting phase transition and transformation to nonbilayer phases are also all dependent on water content. Although there have been various studies of saturated lipids mixed with cholesterol<sup>6</sup> and/or DNA,<sup>7</sup> there have been relatively few studies of lipid bilayers of unsaturated lipids. In particular, x-ray diffraction studies of dioleoylphosphatidylcholine (DOPC) bilayers have previously focused on either low hydration<sup>8</sup> or full hydration<sup>9</sup> while Hristova and White<sup>10</sup> have studied DOPC bilayers at intermediate levels of hydration.

In this letter we report on the structural changes of oriented DOPC multibilayers induced by hydration. In this study, an experimental setup that allows for *in situ* energy dispersive x-ray diffraction (EDXD) experiments with a precise control of relative humidity (RH) and temperature was used. The sample chamber, designed to overcome the experimental inadequacy that had previously led to the vapor-pressure paradox,<sup>11</sup> is elsewhere described.<sup>12</sup> In our investigation, we took specific advantage of using highly aligned lipid multibilayers which allow for more accurate diffraction analyses with respect to liposomic dispersions and are excellent model systems of biological membranes.<sup>13</sup>

DOPC was purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Oriented DOPC multibilayer stacks were prepared by depositing 1 mg of lipid onto a flat freshly cleaved silicon wafer by evaporating from an isopropanol solution. After drying under vacuum for 24 h, the sample was transferred to the hydration chamber. A single diffraction angle  $\theta=1.06^\circ$  was fixed that allowed to cover simultaneously an overall region of the reciprocal space  $0.092 < q < 0.9 \text{ \AA}^{-1}$  ( $q = \text{const} \times E \times \sin \theta$ ,  $\text{const} = 1.014 \text{ \AA}^{-1} \text{ keV}^{-1}$ ).

DOPC multibilayers were hydrated through vapor over the hydration range of 0.45-1 RH at constant temperature

( $T=300 \text{ K}$ ). In this region of the phase diagram ( $0.45 < \text{RH} < 1$ ), DOPC membranes are in the liquid-crystalline  $L_\alpha$  phase where the hydrocarbon chains are melted,<sup>14</sup> while for  $\text{RH} < 0.45$  DOPC membranes are assembled in a three-dimensional rhombohedral  $R$  phase.<sup>15</sup> Since we were particularly interested in the hydration-induced structural changes of the liquid-crystalline  $L_\alpha$  phase, we performed EDXD experiments for  $\text{RH} > 0.45$ .

For  $0.45 < \text{RH} < 0.7$  (data not reported) we did not observe any appreciable structural change. At  $\text{RH}=0.7$ , the EDXD pattern of DOPC (not reported) exhibited five orders of sharp Bragg peaks ( $00l$ ) indicating a high degree of translational order along the normal to the lipid bilayer and a lamellar periodicity  $d=2\pi/q_{001}=48.2 \text{ \AA}$ . This value was in close agreement with those already reported by Caminiti *et al.*<sup>16</sup> The electron density profile (EDP)  $\Delta\rho(z)$  along the normal to the bilayers  $z$  was calculated as a Fourier sum of cosine terms as

$$\Delta\rho = \frac{\rho(z) - \langle\rho\rangle}{[\langle\rho^2(z)\rangle - \langle\rho\rangle^2]^{1/2}} = \sum_{l=1}^N F_l \cos\left(2\pi l \frac{z}{d}\right), \quad (1)$$

where  $\rho(z)$  is the electron density,  $\langle\rho\rangle$  is its average value,  $N$  is the highest order of the fundamental reflection observed in the EDXD pattern,  $F_l$  is the form factor for the ( $00l$ ) reflection, and  $d$  is the lamellar periodicity along the normal to the lipid bilayer consisting of one lipid bilayer and one water layer. The EDP was calculated with the appropriate phases ( $--++$ ).<sup>17</sup>

Figure 1 shows the EDP along the normal to DOPC bilayers. The electron densities of the head groups,  $\rho_H$ , and hydrocarbon tails,  $\rho_C$ , are defined relative to the methylene electron density  $\rho_{\text{CH}_2}$  (Fig. 1, continuous line). According to recent definitions,<sup>18</sup> some water interacting intimately with the lipid membrane should be considered as a part of the structure itself. As a result, the head group size  $d_H$  can be estimated from the full width at half maximum (FWHM) of the Gaussian representing the head group (Fig. 1). As a result, the bilayer thickness is defined as the maximal thickness occupied by lipids, thereby including water molecules associated with the hydration shell of lipid head groups ( $d_B = d_{HH} + d_H$ ).<sup>18</sup> According to geometric considerations, further structural parameters are straightforward to be derived: the hydrocarbon core (HC) thickness  $d_C = (d_{HH} - d_H)$  and the

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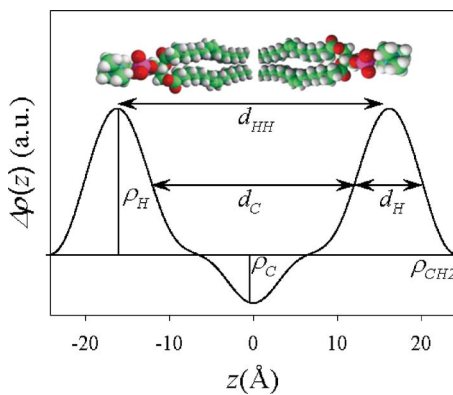


FIG. 1. (Color online) Electron density profile along the normal to DOPC bilayers at RH=0.7. The distance between the two electron density maxima defines the distance between the polar head groups of DOPC molecules, while the head group size  $d_H$  can be estimated from the FWHM of the Gaussian representing the head group. The atoms of the DOPC molecules are color coded: H (white), C (green), N (cyan), P (pink), and O (red).

thickness of the interbilayer water region,  $d_w = d - d_B$ .<sup>18</sup>

Figure 2 shows the changes of structural parameters of DOPC bilayers as a function of RH. Two distinct regimes of hydration were identified. In the first regime (RH < 0.94), the structure of DOPC bilayers was poorly sensitive to changes in hydration, while in the second one (0.94 < RH < 1), distinct structural changes were observed. The lamellar  $d$  spacing and the thickness of the water layer,  $d_w$  [Fig. 2, panels (a)

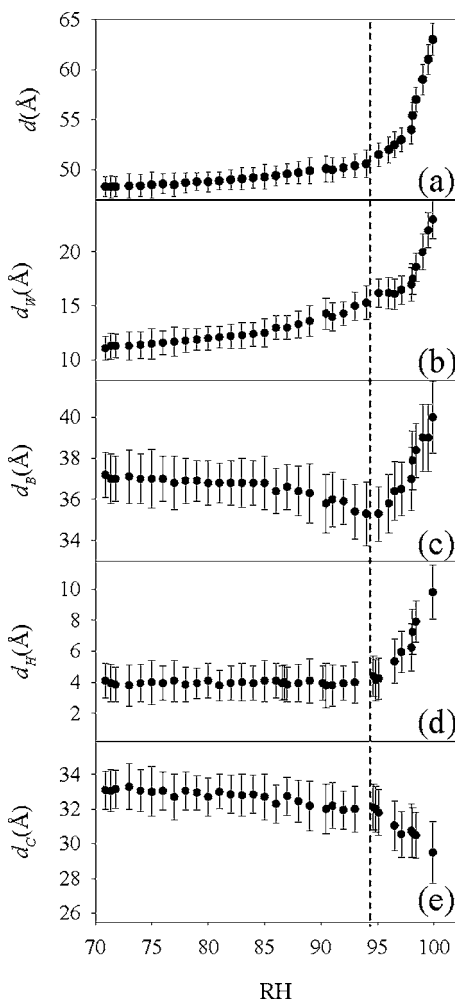


FIG. 2. Structural parameters of DOPC bilayers as a function of RH.

and (b), respectively], were found to swell almost linearly with RH up to RH ~ 0.94 when an abrupt increase was detected. On the contrary, the membrane thickness  $d_B$  [Fig. 2, panel (c)], firstly decreased, passed through a minimum at RH ~ 0.94, and then reached its maximum expansion at full hydration ( $d_B \sim 40$  Å). In the first regime, the head group size  $d_H$  was not influenced, within experimental errors, by changes in hydration [ $d_H \sim 4$  Å, Fig. 2, panel (d)]. In the second regime, we found a strong expansion of the DOPC head group size up to RH=1 ( $d_H \sim 9.8$  Å). This value was found to be in excellent agreement with those reported, at full hydration, by McIntosh and Simon<sup>19</sup> (10 Å) and by Tristram-Nagle *et al.* ( $9 \pm 1.2$  Å).<sup>9</sup> Structural analysis showed the protrusion of DOPC head groups toward the interbilayer water region that accompanies increases in hydration. Structural evolution of  $d_C$  clarified how the hydrophobic core of DOPC bilayers changed with hydration [Fig. 2, panel (e)]. In the first regime, flexibility of DOPC chains was slightly modified by hydration with reduction in HC thickness being less than 1 Å. At hydrations higher than RH=0.94, a marked thinning of the HC occurred as expected from the increased thermal motion of acyl chains that accompanies increased hydration. With further hydration, polar head groups are associated with more water that enlarges the cross-sectional area in the polar head group region, leaving an empty space in the hydrophobic region. The empty space is instantly filled up by flexible hydrocarbon chains, causing the bilayer thinning by hydration.

Our structural results raise the following basic question: Which are the molecular mechanisms underlying experimental observations? Previously, Hristova and White<sup>10</sup> reported a study on the effect of hydration on the HC structure of DOPC bilayers and proposed the existence of a distinctive structural change occurring upon completion of the DOPC hydration shell ( $d \sim 50$  Å; RH > 0.93). Nevertheless, since the observed break in the Bragg spacing curve as a function of adsorbed waters also coincided with a change in the method of hydration,<sup>10</sup> there was a concrete possibility, discussed by the authors, that it was an experimental artifact due to the change of the hydration protocol. In the present investigation, we found a marked structural change (Fig. 2) occurring at RH ~ 0.94 when the lamellar  $d$  spacing was  $d \sim 50.5$  Å. As is evident, our findings confirmed the suggestions by Hristova and White. What are completely different in our EDXD experiments are that (i) a single lipid sample was employed, (ii) a single hydration protocol was used, and (iii) distinct hydrations were achieved directly inside the hydration chamber. Thus, we could unambiguously prove the existence of the hydration-induced structural change of DOPC bilayers previously hypothesized by Hristova and White. Since *in situ* EDXD measurements allowed a very high density sampling of the structural changes of the DOPC bilayer (Fig. 2), we could also clarify and detail the effect of hydration on the structure of DOPC bilayers. It is thought that, at low hydration, lipid molecules interact with each other by electrostatic interactions between the head groups. In the case of neutral DOPC, the phosphate group, which is linked to the glycerol backbone, has a negative charge while the choline group, which constitutes the free end of the head group, has a net positive charge. Upon hydration, the interaction between the lipid head groups in the starting almost dry state is replaced by stronger water head group H bonds between water and lipids. In fully hydrated membranes in the

$L_\alpha$  phase, water molecules interact with the lipid head groups and form bridges among them. We found that, for  $RH < 0.94$ , the head group size along the normal to lipid bilayer is small ( $d_H \sim 4$  Å). This finding indicates that P and N atoms lie in the plane of the membrane. This spatial organization of the  $P^-$  and  $N^+$  atomic groups may allow pairs of DOPC molecules to interact with each other via  $P^- \cdots N^+$  and  $N^+ \cdots P^-$  double pairs. For hydrations higher than  $RH=0.94$ , we found that the head group size along the normal to DOPC bilayer increases up to  $d_H \sim 9.8$  Å. It is known that, upon hydration, water molecules fill the hydration shell of DOPC molecules, thereby reducing the electrostatic interactions between  $P^-$  and  $N^+$  atomic groups. Water molecules are polarized at the lipid-water interface and align their dipole moments toward the water bulk region thus creating a positive potential in the bilayer interior (where P atoms are located).  $N^+$  atomic groups may therefore protrude toward the interbilayer water region to align the head group dipole moment ( $P^- \rightarrow N^+$  vector) against the water dipole moment. In a recent simulation on PC bilayers, Saiz and Klein<sup>20</sup> found that  $P^-$  atoms are located closer to the membrane interior than  $N^+$  atoms. Thus, we suggest that the microscopic origin of the observed swelling in the head group size [Fig. 2, panel (d)] may be the organization at the interface of the polar head groups and the counteracting effect of the polarized hydrating water molecules.

In conclusion we have investigated the effect of hydration on the structure of DOPC bilayers. We have confirmed the existence of a distinctive structural change occurring at  $RH \sim 0.94$ . On the basis of the most accredited conclusions of dynamic simulations carried out on PC bilayers, we pro-

pose that such a marked structural change is likely due to the protrusion of the DOPC head groups towards the interbilayer water region.

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