The Lamellar Repeat Distance of Phospholipid Bilayers in Excess H_2O and D_2O. A Small-Angle X-Ray Scattering Study

Yukihiro Kobayashi and Kazuhiro Fukada*
Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University, 1-1 Minamiosawa, Hachiohji, Tokyo 192-0397

(Received July 6, 1998; CL-980507)

The lamellar repeat distance of multilamellar stack of phosphatidylcholine membranes in excess H_2O and D_2O was determined by small-angle x-ray scattering measurements. When the lipids are in gel-state, the repeat distances in H_2O and in D_2O are almost the same. When the lipids are in liquid crystalline state, on the contrary, the repeat distances in D_2O decrease by ca. 1 Å compared with that in H_2O. It is confirmed that the decrease of the repeat distance is due to the decrease of the water layer thickness.

The multilamellar stack of lipid bilayers in excess water is a good model system for the study of forces acting between large parallel plates. As the forces acting between lipid bilayers, van der Waals, electrostatic, and hydration forces had been considered. Furthermore, with the apparent failure of models based on purely electrostatic and hydration interactions to explain the repulsive forces between lipid membranes, attention has recently focused on the role of thermal fluctuation interaction, e.g., undulation force.[1] In the multilamellar systems, these forces compete with each other, and the net attractive and repulsive forces determine the bilayer-bilayer separation, i.e., water layer thickness. These forces may vary with the lipid bilayer material and the physical properties of the mediums.[2] In the present study, we compared the temperature dependence of lamellar repeat distance of electrically neutral phosphatidylcholines (PCs) in H_2O and D_2O by measuring small-angle x-ray scattering (SAXS) in aid of understanding the role of the mediums in the above mentioned attractive and repulsive interactions.

The PCs studied here were 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), which were purchased from Avanti polar lipids (Birmingham, AL). D_2O (99.9%) was obtained from ISOTEC, Inc. H_2O was purified with Milli-Q labo (Millipore Co., Bedford, MA). The fully hydrated lipid bilayers were prepared by adding excess amounts of H_2O or D_2O to the dry lipids, where the concentration of the lipids were 30-40 wt%. The swollen lipids were annealed with 5 cycles of freezing in liquid nitrogen and thawing at 50 °C in a hot water bath, and were aged overnight at room temperature. SAXS was measured by a high-resolution small angle x-ray scattering instrument.[3] The lamellar repeat distance, D, was calculated from the peak position of the diffracted x-rays using the Bragg equation. The angular resolution associated with the SAXS measurements are 0.005 degree, which corresponds to ca. ± 0.3 Å in D-spacing in the angular range we measured. Also, the repeatability of the D-spacing was confirmed to be better than 0.4 Å.

The temperature dependence of lamellar repeat distance, D, of DLPC, DMPC, and DPPC in excess H_2O and D_2O is shown in Figure 1. The drastic changes in the D-spacing correspond to the pretransition from the gel phase ($L_{\beta'}$) to the ripple phase ($P_{\beta'}$) and the main transition from $P_{\beta'}$ to the liquid crystalline phase (L_α). It can be seen that the temperatures of pretransition, T_p, and

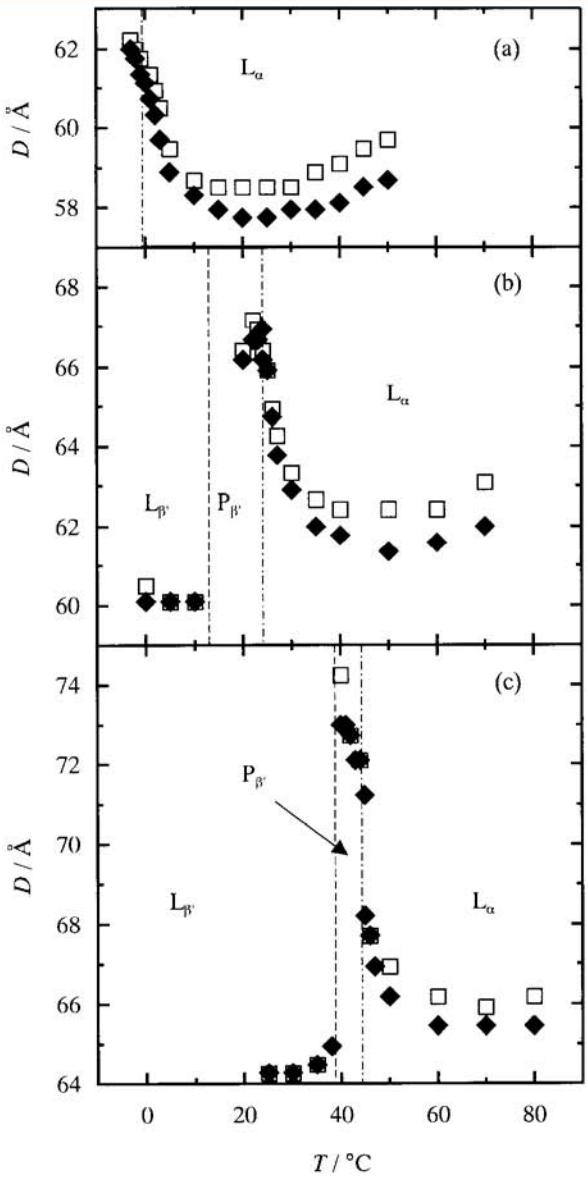

Figure 1. Temperature dependence of lamellar repeat distance, D, of DLPC (a), DMPC (b), and DPPC (c) in excess H_2O (□) and D_2O (◆), respectively. Broken and chain lines indicate the pre- and main transition temperature of the lipids, respectively.

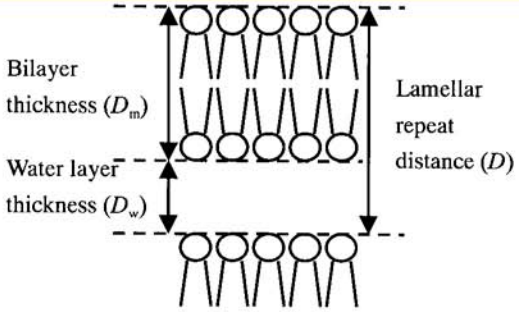

Figure 2. The schematic illustration of a repeat unit in the multilamellar structures of the lipids.

of the main transition, T_m, in H_2O and D_2O are almost the same. The values of T_m (T_p) are –1 °C for DLPC[4], 24 °C (14 °C)[4] for DMPC, and about 45 °C (39 °C) for DPPC, respectively. It is interesting to note that the D-spacing of solid-state PCs (i.e., below T_m) in H_2O and D_2O are almost the same, but the D-spacing in PC-D_2O is smaller than that in PC-H_2O when the lipids are in liquid crystalline state (i.e., above T_m). The difference in the D-spacing between PC-H_2O and PC-D_2O systems in L_α phase varies with temperature from 0 Å (at T_m) to 1 Å (ca. 20 °C above T_m) regardless of the alkyl chain length of lipids. Note that the D-spacing consists of membrane thickness, D_m, and water layer thickness, D_w: $D = D_m + D_w$ (see Figure 2). We determined the D_m in DLPC-H_2O and DLPC-D_2O systems by so called Luzzati method,[5] and confirmed that the values of D_m in H_2O and in D_2O are almost the same (31.5 Å at 20 °C). This result suggests that the decrease of the D-spacing be due to the decrease of D_w.

As the cause for the decrease of D_w in D_2O, the following two possibilities are suggested, 1) the attractive van der Waals force between the lipid bilayers in D_2O is greater than that in H_2O, and 2) the repulsive hydration force and/or the undulation force between the lipid bilayers in D_2O is smaller than that in H_2O. (The electrostatic force can be neglected, since the PC molecules have no net charge.) In the following, we will consider the validity of these two possibilities.

The attractive van der Waals force between lipid bilayers, F_{vdW}, is expressed as

$$F_{vdW} = -\frac{H}{6\pi}\left\{\frac{1}{D_w^{\,3}} - \frac{2}{(D_w + D_m)^3} + \frac{1}{(D_w + 2D_m)^3}\right\} \quad (1)$$

where H is Hamaker constant,[6] which has been estimated as 3-8×10^{-21} J for PCs in H_2O.[7] Based on the Lifshitz theory,[8] one can expect that the Hamaker constant in D_2O is smaller than that in H_2O, since the dielectric constant, ε, of D_2O is smaller than that of H_2O. (The values of ε are 78.1 for D_2O and 78.5 for H_2O at 25 °C, respectively.) This expectation leads to the slight decrease of attractive van der Waals force between lipid bilayers in D_2O than in H_2O in the temperature range studied here, but the experimental results are inconsistent with this expectation. So, the possibility 1) may be discarded as the cause of decrease of D_w in D_2O.

Next, we deal with the repulsive forces between the lipid bilayers. It should be noted here that no difference in the D-spacings between DPPC-H_2O and DPPC-D_2O were observed around 20 °C while D_w of DLPC in D_2O is smaller than that in H_2O at the same temperature (see Figure 1).

Since the hydration force is acting in both gel- and liquid crystalline states, it seems reasonable to suppose that the strength of hydration force does not change so much whether the medium is H_2O or D_2O. The strength of undulation force, on the other hand, strongly depends on the physical state of lipids because of the following reason. The undulation force, F_{undu}, which arises from the entropic confinement of the wave-like motions as two undulating membranes approach each other, is expressed as

$$F_{undu} = \frac{3\pi\,(kT)^2}{64\,\kappa D_w^{\,3}} \quad (2)$$

where κ is the bending elasticity of membrane.[1,9] The values of κ of lipid bilayers were estimated by the micropipet manipulation technique for giant unilamellar vesicles.[10] The estimated κ in the gel phase is at least 34 times larger than the value in the liquid crystalline phase. Hence, one can expect that the undulation force governs the change of bilayer-bilayer separation in the liquid crystalline state of lipids but which is unimportant when the lipids are in gel-state. Our result strongly suggests that the decrease of D_w in D_2O, which observed only when the lipids were in liquid crystalline state, be caused by lowering the contribution of repulsive undulation force between the bilayers.

At present, the relation between the physical properties of D_2O and the strength of undulation force is not clear, but a possible explanation may be that with the change of medium from H_2O to D_2O the interactions between the neighboring lipid molecules in bilayers are strengthened, and which leads the increase of bending elasticity of membranes.

References and Notes

1 J. N. Israelachvili, in "Intermolecular and Surface Forces (Second Edition)," Academic, New York (1991), Chap. 18, p.395.
2 T. Hønger, K. Mortensen, J. H. Ipsen, J. Lemmich, R. Bauer, and O. G. Mouritsen, *Phys. Rev. Lett.*, **74**, 3911 (1994).
3 H. Yoshida, T. Kato, K. Sakamoto, and T. Murata, *Memoirs Fac. Engineer. Tokyo Met. Univ.*, **44**, 4977 (1944).
4 D. Marsh, in "CRC Handbook of Lipid Bilayers," CRC Press, Boca Ranton (1990), Chap. 11.7, p.136.
5 Y. Kobayashi and K. Fukada, *Biochim. Biophys. Acta*, **1371**, 363 (1998).
6 E. J. W. Verwey and J. Th. G. Overbeek, in "Theory of the Stability of Lyophobic Colloids," Elsevier, Amsterdam (1948), p. 102.
7 L. J. Lis, M. McAlister, N. Fuller, R. P. Rand, and V. A. Parsegian, *Biophys. J.*, **37**, 657 (1982).
8 J. N. Israelachvili, in "Intermolecular and Surface Forces (Second Edition)," Academic, New York (1991), Chap. 11, p.189.
9 W. Helfrich, *Z. Naturforsch.*, **33a**, 305 (1978).
10 D. Needham and D. V. Zhelev, in "Vesicles: Surfactant Science Series Vol. 62," ed by M. Rosoff, Marcel Dekker, New York (1996), Chap. 9, p.394.