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Hydration Forces between Phospholipid Bilayers at Subzero Temperatures

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A hydration repulsive force plays an important role in various phenomena. In order to study its temperature dependence, the hydration force of dipalmitoylphosphatidylcholine bilayers was investigated at temperatures below freezing. Using known chemical potential of ice, the strength of the interbilayer repulsive force of dipalmitoylphosphatidylcholine bilayers at subzero temperatures was estimated and X-ray diffraction was used to estimate the interbilayer spacing. An exponentially decaying repulsive force with a decay constant of 0.15 nm was observed. The present subzero temperature data are slightly greater than those measured at temperatures about 20 °C.

Keywords: hydration force; phosphatidylcholine: X-ray diffraction; ice; osmotic pressure

INTRODUCTION

Repulsive forces play an important role in various phenomena, such as coagulation of colloids, stability of lyotropic liquid crystals, fusion of lipid vesicles and biological membranes, etc. In the case of lipid bilayers, many experimental studies have revealed the repulsive force between the lipid bilayers decays exponentially as a function of the distance between bilayers. The force is called hydration repulsive force [1].

The physical origin of the hydration repulsive force has been controversial. Several theories have proposed for the mechanism of the force. These theories can be classified into two groups. One of these theories explains the hydration force in terms of the non-random orientation of water molecules near the surface [2]. The second of these theories states that the origin of the force is due to a combination of mode and amplitude of thermal motion of the surface [3]. Both theories predict that the force decays exponentially with increasing distance from the bilayer surface. The first theory predicts stronger repulsion at low temperature, because the forces are mainly due to water structure. By contrast, the second theory predicts weaker repulsion at low temperature, because the thermal motion is reduced at low temperature. Thus, it is of interest to study the sign of temperature dependence of the hydration forces.

Using nuclear magnetic resonance (NMR) technique, Yan *et al.* [4] have carried out the first measurement of the hydration force of phospholipid multilamellar systems at subzero temperatures to test the theories. The principle of their measurement is based on the following facts: In frozen lipid-water systems, because water molecules in the interbilayer (interlamellar) region are unfrozen, crystalline ice formed in extralamellar region is in equilibrium with the interbilayer (interlamellar) water molecules. In other words, in a frozen

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phospholipid sample, the repulsive hydration force is balanced with the osmotic pressure produced by ice formed in extralammellar region. The osmotic pressure can be calculated from the chemical potential of ice as a function of temperature. Yan *et al.* [4] have compared the force-distance data at subzero temperatures obtained this method with the data obtained at temperatures about 20 °C to discuss the sign of temperature dependence of the hydration force. However, in above NMR method, the interbilayer (interlamellar) spacings are estimated indirectly. Yan *et al.* [4] have estimated the distance between the bilayers from the fraction remaining unfrozen water determined by its contribution to the NMR signal. Thus, direct measurements of the spacings are required for detailed discussion of temperature dependence of the hydration force.

In this study, we re-examined the interbilayer force of dipalmitoylphosphatidylcholine (DPPC) bilayers, one of the most extensive studied phospholipids, at subzero temperatures. A particular feature of our study was to estimate the interbilayer spacing directly from X-ray diffraction data. For this purpose, we reconstructed the electron density profiles of DPPC at subzero temperatures. The hydration force for DPPC bilayer measured at subzero temperatures in this study is slightly greater than that measured at temperatures about 20 °C [5]

MATERIALS AND METHODS

The dry powder of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) was purchased from Avanti Polar Lipids. The lipid was found to be at least 99 % pure by thin-layer chromatography. No further purification was carried out. Suspensions of fully hydrated multilamellar DPPC vesicles were prepared by mixing dry DPPC with

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approximately the same mass of distilled water. The samples were kept at about 60 °C for 30 min and then cooled down to 20 °C. This cycle of heating and cooling was repeated to get homogeneously hydrated samples.

X-ray diffraction studies were performed at station 8.2 of the Synchrotron Radiation Source at Daresbury Laboratory, UK. Details of the station set-up have been published elsewhere [6]. Samples were mounted in a slot 1 x 5 mm cut in a 1 mm thick copper plate that was sandwiched between a pair of thin mica sheets. The sandwich was clamped directly onto a silver block of a cryomicroscope stage.

RESULTS AND DISCUSSION

X-ray diffraction patterns of a fully hydrated multilamellar DPPC sample recorded during heating from - 50 °C are shown in Fig.1. Figure 2 represents the temperature dependence of lamellar spacings determined from the peak positions of the diffraction patterns. The lamellar spacings gradually increase from about - 35 °C and above 0 °C, the lamellar spacings are almost constant.

In order to estimate the interbilayer spacing, we reconstructed electron density profiles according to a following procedure: First, the observed intensities of the lamellar reflections were converted to structure amplitudes according to standard method [7]. Phases of the reflections were determined from the analysis of plots of structure amplitudes as a function of the reciprocal space, using the Shannon sampling theorem [8] (Inset of Fig.2). The electron density profiles at several temperatures are shown in Fig.3.

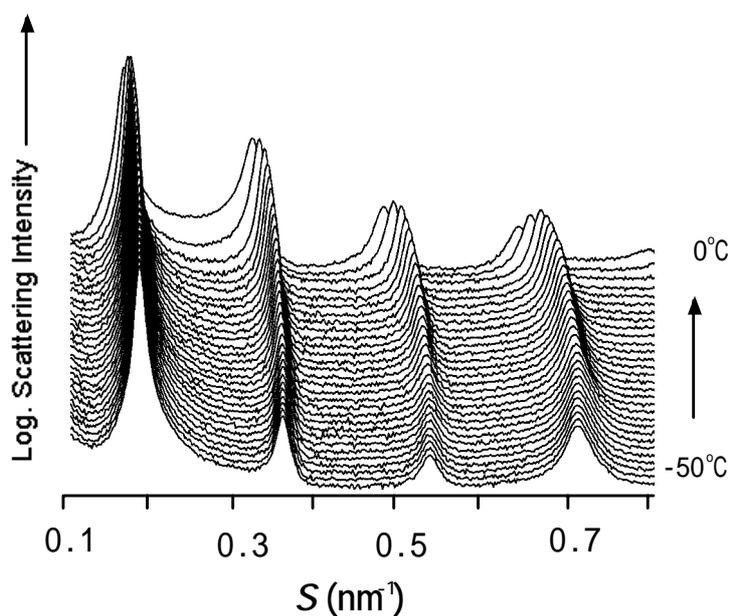


FIGURE 1 Plot of X-ray diffraction patterns of DPPC undergoing a heating scan rate of 1.8 °C as a function of reciprocal space ($S = 2\sin\theta/\lambda$, $2\theta =$ scattering angle, $\lambda =$ wavelength of X-rays).

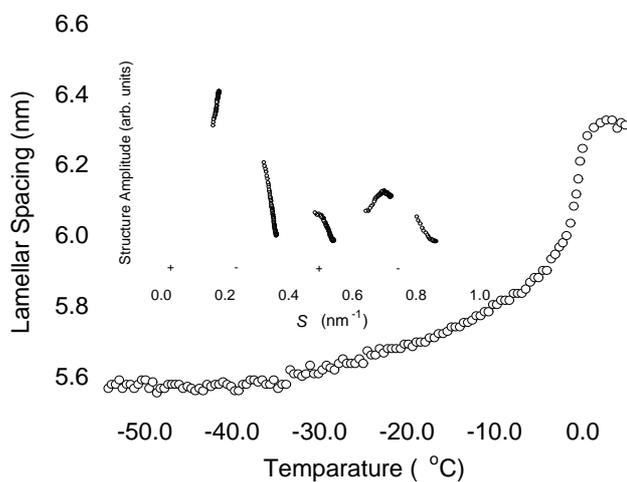


FIGURE 2 Lamellar spacing vs. temperature. **Inset:** Structure amplitude of DPPC vs. reciprocal space. The value at $S = 0$ was estimated by the method proposed by King and Worthington [12]. The solid curve was calculated using the Shannon sampling theory.

At this resolution, the peaks in the electron density profiles correspond to near the center of the phosphatidylcholine (PC) head group [9]. Using Space-filling molecular model, McIntosh and Simon [5] have estimated that the distance from the center to the outer edge of the PC head group is about 0.5 nm. Based upon this estimation, they have defined the bilayer thickness as the distance between high-density peaks across the bilayer plus 1.0 nm. We also used this definition of the bilayer thickness and calculated the interbilayer spacing by subtracting the bilayer thickness from the lamellar spacing.

The forces between the bilayers at subzero temperatures can be estimated from the chemical potential of ice [4]. The chemical potential of ice is calculated principally, based on the experimental data for the difference of the specific heat of ice and undercooled water. However, the experimental data at all temperatures we measured in this study are not available. Thus, we calculated them using an approximate equation, so-called the Hoffman's equation [10].

According to the procedure described above, we obtained the relation between the interbilayer repulsive force and the interbilayer distance of DPPC at subzero temperatures (Fig.4). The repulsive force decays exponentially and the decay constant is about 0.15 nm. The

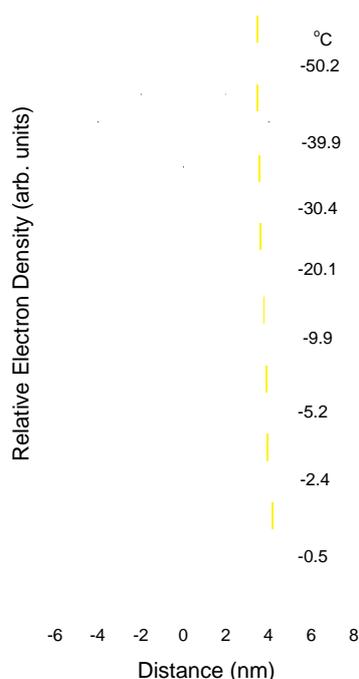


FIGURE 3 Electron density profiles of DPPC at subzero temperatures.

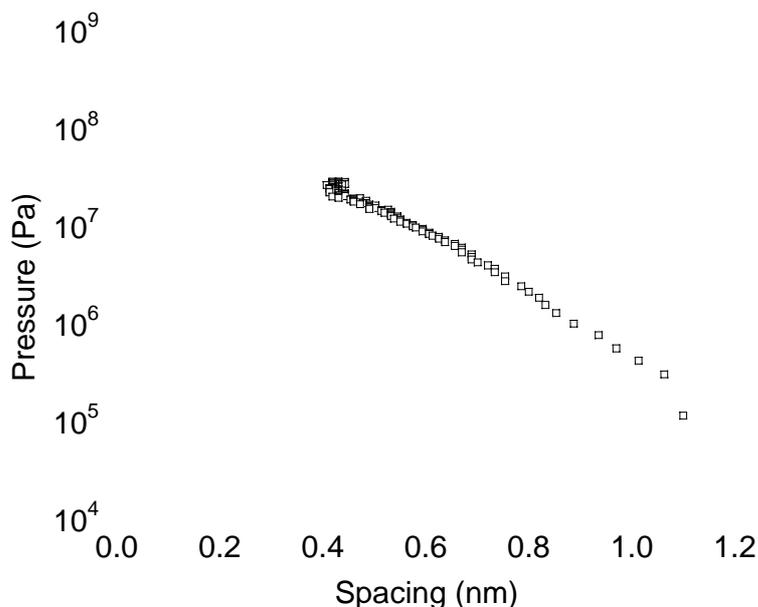


FIGURE 4 Interbilayer force vs. the distance of interbilayer of DPPC at subzero temperatures. The continuous line represents a fit a data by McIntosh and Simon [5] for DPPC at room temperature.

present data of the repulsive force at subzero temperature are slightly greater than those measured at about 20 °C by McIntosh and Simon [5] who have measured the interbilayer repulsive force by applying the osmotic stress when DPPC is incubated in aqueous solution of neutral high molecular weight polymer.

The attractive force due to the van der Waals interaction also contributes the total forces between phospholipid bilayers and the strength of van der Waals interaction depends on temperature [1]. As estimated by LeNeveu *et al.* [11], however, the attractive force due to the van der Waals interaction is of negligible magnitude compared to the hydration repulsive force below the equilibrium interbilayer

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separation, i.e., the interbilayer spacing without an osmotic pressure. Therefore, our data suggest that the hydration repulsive force becomes stronger at low temperatures. The present study supports the theory based on the water structure for the hydration repulsive force [2].

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