

Hydration kinetics of oriented lipid membranes investigated by energy dispersive x-ray diffraction

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Energy dispersive x-ray diffraction was applied to investigate the hydration kinetics of highly aligned 1,2-dioleoyl-3-trimethylammonium-propane membrane system. First, the adsorption of water into the lipid film results in a continuous gain of interbilayer spatial coherence until a maximum is reached. Further, adsorbed water molecules behave as bulk water promoting loss of spatial coherence and leading to a progressive lowering and broadening of diffraction peaks. The possible molecular origin of this phenomenon is discussed. © 2004 American Institute of Physics. [DOI: 10.1063/1.1785855]

A fundamental problem in membrane biophysics is lipid hydration and its effect on the structure of lipid bilayers.^{1,2} Experimental and theoretical studies have shown that lipid hydration strongly affects molecular interactions in the head-group region^{3,4} and results in the development of interbilayer interactions known as "hydration forces."^{5,6} Such forces have been investigated using a number of different and complementary methods and were identified as the dominant non-specific short-range interactions between lipid bilayers. The origin of the hydration force is still not completely understood. It is sometimes attributed to normal motion of the surface, either individual molecular motion or surface undulations.⁶ A more widely accepted view is that it is due to ordering of water at the surface, which propagates out from the surface with decreasing strength.⁷

In particular, a great deal of research has been addressed to the understanding of the spontaneous water uptake by lipid membranes.⁸ Unfortunately, although a basic phenomenon in biophysics and related biological sciences, there is no satisfactory interpretation of the molecular mechanisms and the physical principles driving the hydration of lipids so far.

In this letter, we report our preliminary results on the hydration kinetics of solid-supported 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) membranes investigated by means of energy dispersive x-ray diffraction (EDXD). Information about this nonconventional technique has been given elsewhere.⁹ EDXD appears to be of great interest in studying the kinetic variation of an ordered structure (lipid bilayer) induced by the change of physical and/or chemical parameters.¹⁰ DOTAP was purchased from Avanti Polar Lipids in the lyophilized form and used without further purification. A multilayered stack of highly aligned DOTAP membranes was prepared depositing 1 mg of DOTAP onto a flat substrate (1×1.5 cm piece of freshly cleaved $\langle 100 \rangle$ silicon wafer) by evaporating from an isopropanol solution. After drying under a vacuum for 1 day, the sample was transferred to the x-ray sample chamber constructed according to the indications given by Katsaras.¹¹ Preliminary measurements (data not reported) showed the lipid to be, as expected, in the lamellar liquid-crystalline phase L_α in which the hy-

drophobic chains are conformationally disordered. The best experimental conditions were met at the diffraction angle $\theta = 0.3^\circ$ corresponding to the maximum diffracted intensity. The lipid film was hydrated at $T = 300$ K from a water-saturated vapor as previously described.¹⁰ Closing the chamber mylar windows, the adsorption of water onto the sample immediately proceeds. The uptake of water into multilayered DOTAP film exposed to water vapor was followed as a time function. For kinetics experiments, the diffraction intensity was collected and automatically stored every 4 s.

At $t = 0$, an interlamellar separation $d = 47.5$ Å was calculated from the position of the Bragg peak maximum by applying the Bragg's law ($d = 2\pi/q$). Upon progressive hydration, the adsorbed water, permeating vertically through the lipid film, resulted in the swelling of DOTAP membranes. Water molecules could also diffuse through defects between the layers. Figure 1 shows the temporal kinetics of water adsorption by DOTAP film resulting in a continuous swelling of interlamellar separation d . Besides, the slow swelling kinetics is an expected result, since water penetration is greatly impeded by the hydrophobic chains. Previous spectroscopic studies confirm that transport of water through these well-ordered densely packed films is a slow process.¹² The interlamellar separation temporal evolution was fitted with the following double exponential model

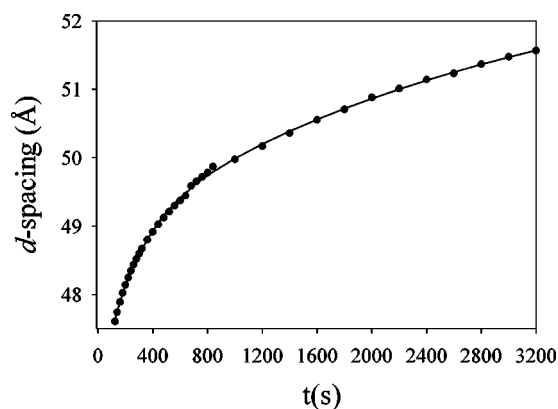


FIG. 1. Temporal evolution of the interlamellar separation d of DOTAP membrane system. Water molecules are incorporated between lipid bilayers and the lipid swells monotonously in the investigated temporal range. The solid line is the best fit to the data [obtained applying Eq. (1)].

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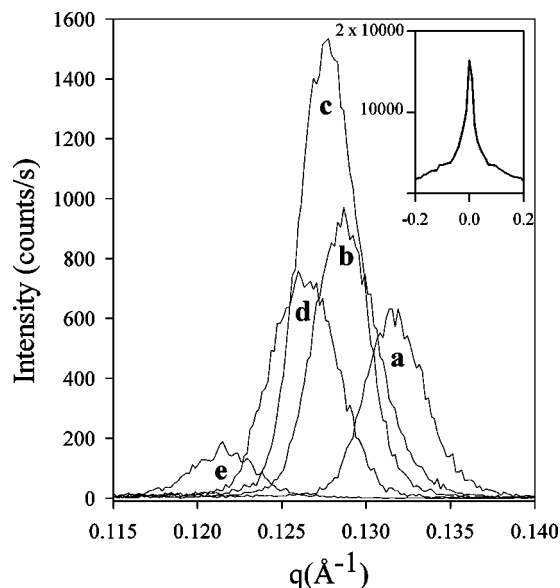


FIG. 2. Representative EDXD patterns collected at $t=0$ s (a), 340 s (b), 480 s (c), 1200 s (d), and 3200 s (e). At the beginning of the hydration kinetics, the water adsorption increases the interbilayer spatial coherence leading the diffracted intensity to a maximum value (c). Further adsorbed water molecules behave as bulk water reducing the correlation length of the domains within which the sample scatters coherently. This leads to a progressive lowering and broadening of diffraction peaks (d, e). A rocking curve at the q position of the first Bragg peak (a) is shown in the inset. The full width at half maximum of 0.03° is determined by instrumental resolution, underlining the high orientational order of the sample.

$$d = d_0 + d_1(1 - e^{-(t/\tau_1)}) + d_2(1 - e^{-(t/\tau_2)}). \quad (1)$$

Two relaxation components were revealed: A faster process, proceeding within hundreds of seconds ($\tau_1 \sim 240$ s), and a slower process, on the time scale of a few thousands of seconds ($\tau_2 \sim 3000$ s). In Fig. 2, the effect of hydration on the diffracted intensity is shown. By careful analysis of the EDXD spectra, the water adsorption phenomenon appears to be rather interesting. In fact, while the lipid swells monotonously with increasing time, diffraction intensity shows a dissimilar behavior. For the sake of clarity, the integral value of the peak region as a time function is reported in Fig. 3. As evident the integral area firstly increases, passes through a pronounced maximum at $t \sim 480$ s ($d \sim 49.2$ Å) and subsequently falls off until a plateau is reached. In multilamellar samples, diffraction peaks represent interbilayer coherence, and the amplitude of diffraction maximum is a powerful tool to evaluate the ordering or disordering effect of adsorbed

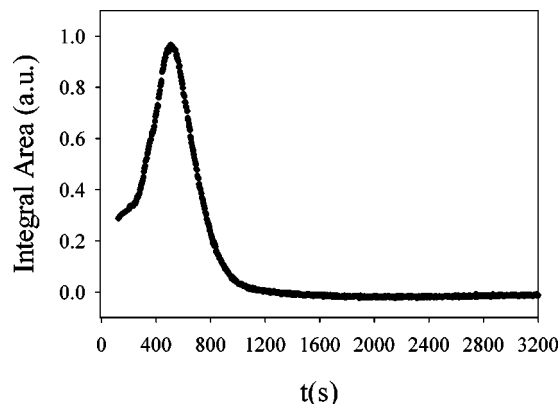


FIG. 3. Integral area of the region peak as a time function.

water molecules. Thus, what should be clear here is that not all the adsorbed water molecules behave the same. Indeed, EDXD patterns unambiguously indicate that water molecules interacting first, and most intimately, with a lipid have a strong influence on the interbilayer interactions and seem to stabilize the overall structure of the system until a maximum. After the highest degree of spatial coherence is reached, further adsorbed water molecules behave as bulk water reducing the correlation length of the domains within which the sample scatters coherently. Indeed, bulk water molecules have a liquid-like disorder resulting in dynamical variations of the repeating distance d in a multilayer. As a result of such motions, the multilayered structure fluctuates from instant to instant and such fluctuations are likely to contribute to stacking disorder. Lattice disorder and undulations increase and the loss of spatial coherence leads to a progressive lowering and broadening of diffraction peaks. The wide peak labeled as e in Fig. 2 clearly shows that the intermembrane coherence extends less far, upon hydration, for disorder of the second kind. On the whole, our EDXD data show that the first water binding to the polar groups of the lipid increases the molecular order degree of the system and that the disordering effects enabled by the bulk water dominate over ordering effects of strictly bound water.

Which are the physicochemical mechanism underlying the observed intriguing phenomenon? About its origin, some hypotheses can be made. Recently, experimental results have clarified the exact nature of liquid water in the vicinity of bilayers.¹³ At low interlamellar separation, water molecules were found to be strongly ordered with their dipoles aligned against the bilayer dipole whereas, at larger lamellar separation, most of the interlamellar water behaved as bulk water. Furthermore, it was elsewhere proposed that the first adsorbed water molecules can modulate the molecular arrangement of lipids with the headgroups locked into a highly ordered and stable structure.¹⁴ Headgroup-headgroup interactions in the starting almost dry state could be replaced by stronger water-headgroups H bonds between water and lipid.

In principle, both mechanisms could be responsible for the above discussed starting gain and following loss of interbilayer spatial coherence. Diffraction data could also be indicative of a progressive structural change upon completion of the first hydration shell of the lipid as reported by Hristova and White in the case of dioleoylphosphocoline bilayers.¹⁵ The small peak area at a reduced level of hydration could also be a consequence of disordering due to defects between bilayers. Upon hydration, the membrane systems could be stabilized by the adsorption of water molecules reducing such defects.

In conclusion, our preliminary measurements have shown the existence of hydration-induced relaxation processes with a cooperative dynamics of water plus DOTAP system. In this sense, the chemical nature of the bilayer surface may be the rationale to govern the ordering of the multilayered stack of membranes. Experiments aimed at elucidating the precise influence of the lipid headgroup on the hydration kinetics of lipid membrane systems are currently in progress in our laboratory.

Furthermore, EDXD has revealed itself as a powerful tool to follow the kinetics of spontaneous hydration of oriented membranes. The time resolution of the order of seconds gives the opportunity to study hydration kinetics in detail.

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