

Pressure Induced Hydration Dynamics of Membranes

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Pressure-jump initiated time-resolved x-ray diffraction studies of dynamics of the hydration of the hexagonal phase in biological membranes show that (i) the relaxation of the unit cell spacing is nonexponential in time; and (ii) the Bragg peaks shift smoothly to their final positions without significant broadening or loss in crystalline order. This suggests that the hydration is not diffusion limited but occurs via a rather homogeneous swelling of the whole lattice, described by power law kinetics with an exponent $\beta = 1.3 \pm 0.2$.

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The availability of high brightness x-ray synchrotron sources and a new generation of detectors that acquire complete 2D Bragg diffraction patterns at video rates [1,2] enables the direct structural study of the dynamics of lyotropic liquid crystalline systems on millisecond time scales. This allows study of diffusion and water transport in these topologically complex systems which are of interest not only to the biophysics community, but also generally to those interested in fluid invasion into porous media with nanometer sized pores.

Dispersions of biological lipids in water exhibit a rich variety of liquid crystalline phases in which membranes form periodic lattices in either 1, 2, or 3 dimensions [3]. The inverted hexagonal (H_{II}) phase [Fig. 1(a), inset] is one of the simpler nonlamellar phases and has relevance to biological processes as well [4]. This H_{II} phase is formed by a periodic lattice of cylindrical pores of water ~ 40 Å in diameter, each surrounded by a monolayer of lipid molecules. Detailed studies [5] done on such systems in equilibrium have shown [6,7] that the thermodynamics of such nonlamellar phases can be understood as a result of a *frustration* between the opposing requirements that membranes have to curl to satisfy curvature requirements on the one hand but also to maintain constant bilayer thickness on the other. This frustration results in extreme sensitivity of the H_{II} unit cell spacing a to changes in both temperature and pressure [8]. Application of hydrostatic pressure in the presence of excess water [9] leads to an *increase* in a , due primarily to an increase in the diameter of the water core, and hence, in the number of water molecules n_w associated with each lipid molecule [8,10]. A pressure jump can therefore be used to induce a sudden change in the chemical potential of water in order to drive water into or out of the H_{II} domains. The dynamics of water uptake/release can then be examined.

Time-resolved studies.—Time-resolved x-ray diffraction studies of the H_{II} phase were done at beamline X9A at the Brookhaven National Synchrotron Light Source, using pressure jumps in the range 0.1–2 kbar over a tem-

perature range of 280–350 K. The beam had an intensity of about 10^{11} photons/s in a ~ 500 μm spot, and an energy of $E = 8$ keV, with a resolution of $\Delta E/E \sim 10^{-4}$. Samples of about 50 mg of material were contained in a temperature-controlled high-pressure cell with beryllium windows. Unit cell spacings were obtained from positions

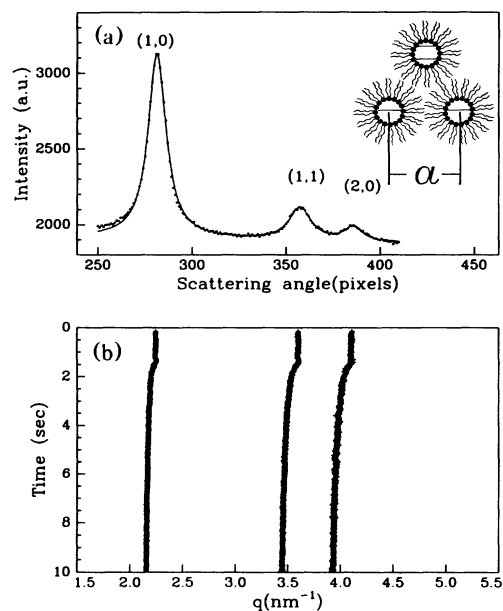


FIG. 1. (a) A fit to the integrated diffraction pattern. Fitting the Bragg diffraction peaks to Lorentzian line shapes allowed for determination of the positions, peak widths, and areas of the peaks as a function of time. For the pattern indicated, the intercylinder spacing $a = 69.1$ Å; analysis of the peak widths yields a variance $\Delta a = 0.6 \pm 0.2$ Å which characterizes the crystalline disorder. (b) Bragg peak positions as a function of time. Integrations along the equatorial plane of the x-ray data obtained from time-resolved x-ray diffraction. An upward pressure jump from 100 bars to 1200 bars at a temperature of 318 ± 4 K occurs about 2 s after the x-ray shutter is opened.

of the Bragg peaks with calibration to silver stearate. The instrumental resolution (FWHM) for the scattering angle was $\Delta\theta \sim 1 \times 10^{-3}$ rad. Fast pressure jumps with a rise time ~ 7 ms were obtained by the opening of a high-pressure valve driven by a computer-actuated pneumatic piston, similar to the method of Caffrey and co-workers [11]. The pressure jumps resulted in small temperature changes (~ 2 K for a 0.5 kbar jump) as measured by a fast response thermocouple embedded within the sample. A home-built intensified x-ray detector system based on a two-dimensional video rate CCD imager running in non-interlaced mode (with a frame time of 16 ms) was used to obtain more than 10^6 x-ray diffraction patterns in order to investigate both pressure-induced phase transitions (to be reported elsewhere), as well as the pressure-induced changes in the structure of a single phase reported in this Letter.

Sample preparation.— H_{II} phases formed by aqueous dispersions of the lipid DOPE (dioleoyl-phosphatidyl-ethanolamine) were investigated, both with and without addition of a small amount (~ 0.05 w/w) of dodecane. The presence of the alkane extends the H_{II} phase boundary, permitting studies over a much larger pressure range [4]. All experiments were performed on hydrated "powder" samples, which contain a large number of randomly oriented crystallites of several microns size. Samples were prepared by mechanically mixing ~ 200 mg of lipid (or lipid+alkane) with an equal amount of water. Even with mechanical mixing, full hydration of the liquid crystalline domains can take many days. Samples that were not fully hydrated showed broad Bragg diffraction peaks, indicative of a wide distribution in water concentration caused by the slow diffusion of water through many layers of lipid. Hydration was sped by incubating the sample at 10°C and 1500 bars for several hours. Subsequent experiments were performed at higher temperatures and lower pressures which require a lower concentration of water for full hydration of the liquid crystal. Fully hydrated samples exhibited sharp Bragg diffraction patterns and were used for the pressure jump studies reported here. Samples were checked for degradation by thin-layer chromatography both before and after x-ray exposure.

Results.—Figure 1 shows diffraction from an aqueous DOPE-dodecane sample at 318 K undergoing a pressure jump from 100 to 820 bars. Figure 1(a) shows a representative plot of scattered intensity as a function of the magnitude, q , of the scattering vector. The diffraction peaks can be fitted to Lorentzian profiles with the peak positions yielding the spacing, a , of the hexagonal lattice. Several striking aspects of the data are illustrated in Figs. 1 and 2. (i) The peak positions relax continuously to their final value without significant increase in disorder. Note that the peak widths in any given frame are seen to increase with increasing diffraction order, characteristic of samples in which Bragg peaks are broadened by static lattice disorder ("disorder of the second kind") rather than thermal (Debye-Waller) effects [12]. Analysis

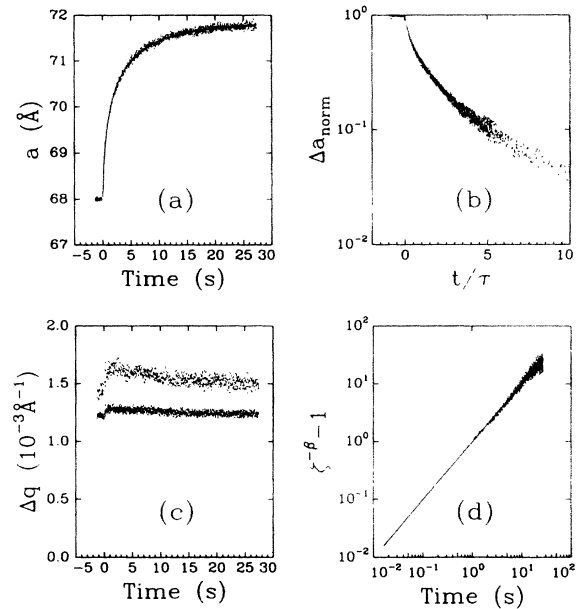


FIG. 2. (a) Unit cell spacing in the H_{II} phase as a function of time. (b) Rescaled plot of Δa_{norm} as a function of time showing the nonexponential nature of the decay. Data shown are from five different pressure jumps ranging in amplitude from 0.7 kbar to 1.3 kbar, with changes in the unit cell spacing ranging from 2 Å to 7 Å and appear to collapse to a single normalized curve. The time axis has been rescaled with a characteristic time constant τ obtained from a fit shown in (d) (see below). (c) Observed peak width as a function of time for the (1,0) and (1,1) peaks of the inverted hexagonal lattice. (d) A log-log plot of the normalized relaxation function shows that the relaxation is strongly nonexponential. Solid line is a fit to a power law form discussed in the text.

of the Lorentzian profile of the peaks, correcting for the finite beam width using standard techniques [12], shows that the density-density correlation function decays as $\langle \rho(0)\rho(r) \rangle \sim e^{-r/\xi}$ with $\xi = 1800 \pm 500$ Å. During the pressure jump shown in Fig. 1, the angular peak width $\Delta\theta$ of the (10) peak increases by $\sim 6 \times 10^{-5}$ rad, much less than the shift in the peak position of 2×10^{-3} rad from its initial to final value. (ii) Immediately following the pressure jump, the relative intensities of the diffraction orders change, indicating a change in molecular arrangement has occurred. These intensity changes are consistent with those observed in equilibrium studies at various hydrations and pressure, suggesting a quasistatic picture in which elastic deformations have relaxed on time scales shorter than 10 ms. On a longer time scale (s), the liquid crystal adjusts its composition (i.e., n_w) in response to the change in the chemical potential of water brought on by the change in pressure. This change in composition is manifest by the change in spacing, a . (iii) The relaxation of the lattice spacing, a , is nonexponential in time [Figs. 2(a) and 2(b)]. Moreover, the data from all the pressure jumps collapse onto a single curve if they are rescaled by a time constant and by an amplitude transform of the

form

$$\Delta a_{\text{norm}} = \frac{a(t) - a(0)}{a(\infty) - a(0)}, \quad (1)$$

where $a(0)$ and $a(\infty)$ are the initial and final lattice spacings which depend only on the initial and final pressures of the jump sequence [Fig. 2(b)].

Given the quasistatic picture, the measurement of a as a function of time, t , can be combined with equilibrium high-pressure dilatometric data [8] to measure the number of water molecules per lipid molecule, $n_w(t)$ [Fig. 2(d)]. n_w is related to a and the radius of the water core, R_w , by a simple geometric relation:

$$n_w = \frac{v_l}{v_w} \frac{2\pi R_w^2}{\sqrt{3}a^2 - 2\pi R_w^2}. \quad (2)$$

The ratio v_l/v_w of the molecular volumes of the lipid and water is known from high-pressure dilatometry measurements [8], and shows that the increase in a under pressure is due to an increase in n_w . $R_w(a, P)$ was found by static x-ray measurements [8]. Measurements of the intrinsic crystalline disorder Δa gives us the variance Δn_w . The quality of our data is such that only the first and second moments of the probability distribution $p(n_w, t)$ can be obtained. Figure 3 shows a plot of $p(n_w, t)$ as a function of time, and shows that the mean value, \bar{n}_w , also relaxes nonexponentially [Fig. 2(c)] while the width of the distribution $p(n_w, t)$ does not change significantly. We may assume that, to a first approximation, the system may be described just by specifying the mean number $\bar{n}_w(t)$ as a function of time. We define a rescaled relaxation function

$$\zeta(t) = \frac{\bar{n}_w(t) - \bar{n}_w(\infty)}{\bar{n}_w(0) - \bar{n}_w(\infty)}. \quad (3)$$

$\zeta(t)$ relaxes from an initial value of 1 to a final value of 0. Nonexponential kinetics are often analyzed empirically by fitting the observed decays to either stretched expo-

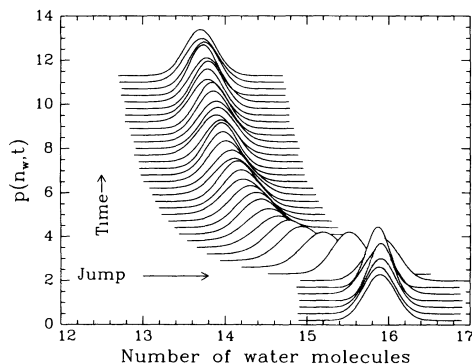


FIG. 3. A time sequence of the probability distribution $p(n_w, t)$ obtained from the measured first and second moments for a pressure jump from 1 kbar to 0.13 kbar. Curves at 240 ms interval have been displaced vertically for clarity. Note that the increase in the width of the distribution is about 10 times smaller than the shift in the average position.

ponential or power laws, particularly in the long time limit. We find that the data may be fitted to the empirical form $\zeta(t) = (1 + t/\tau)^{-1/\beta}$. A plot of $(\zeta^{-\beta} - 1)$ as a function of time appears linear over nearly three decades in time [Fig. 2(d)] [13], as can be seen in Fig. 2(d). All pressure jumps, both upwards and downwards, can be fitted to this functional form with an exponent of $\beta = 1.3 \pm 0.2$. For a sample of a given chemical composition, the characteristic time constant depends on temperature, but is relatively insensitive to the pressure or even the direction of the pressure jumps. In the absence of a microscopic description, the proposed power law decay can only be regarded as a phenomenological characterization of the data, but may serve as a guide to future theoretical work. Studies at shorter times and over larger pressure ranges are needed to improve the error bars in the exponent β , and to observe if there are systematic deviations from the scaled collapse in Fig. 2(b).

We know of no simple model which explains observations (i)–(iii). A straightforward explanation for the observed nonexponential nature of the kinetics is to postulate that the observed relaxation is due to domains of different sizes relaxing with different time constants to the final value of the lattice spacing. Perhaps the simplest model is a two-state model, with the system relaxing from its initial state to the final state. Such a model would give rise to a diffraction pattern in which the scattering pattern at intermediate times is a superposition of initial and final state Bragg patterns. Inspection of Fig. 1 shows that such a superposition is not observed. Extensions of this idea in which each domain relaxes exponentially but independently (“in parallel”) also lead to the prediction that the peak widths following the pressure jump would be much broader than observed.

Power law kinetics arise generically in diffusion problems. Water diffuses in from the outer surface of the crystallite driven by the pressure-induced chemical potential difference. If the observed kinetics are indeed determined by diffusion of water inside the liquid crystal, one would predict that density-density correlation would be reduced, leading to an increase in peak widths. Simulations of such models suggest that the widths of the Bragg peaks should increase by an amount comparable to the shifts in peak position. However, this is not what is observed in Figs. 1 and 2. Sophisticated treatments of concentration changes following a perturbation have been based on dynamics of the type first analyzed by Cahn and Hilliard (for both conserved and unconserved order parameters) [14]. Linearized versions of such treatments invariably lead to exponential kinetics. There have been recent reports of extensions to the Cahn-Hilliard theory to take into account nonlinear effects due because the mobility may depend on the local concentration or on time [15], but applications of this approach to the present problem are unclear at present. The phenomenological characterization of our data presented above may provide a guide to future theoretical work.

One class of models that is consistent with the spirit of the discussion above are *sequential models* of relaxation inspired by spin glass literature, in which water molecules encounter barriers of ever increasing height, with the barrier height being renormalized by the number of water molecules already transported. This model would lead to a swelling of the lattice and is consistent with the observed x-ray diffraction data. Nonexponential kinetics then arise from a distribution of barriers to the entry of water molecules. Such a sequential model assumes that diffusion of water molecules within the crystallites is not the rate limiting process. A uniform swelling of all the cylindrical pores is possible only if all the water cylinders locally have ready access to water. This picture may be difficult to reconcile with electron micrographs of compact H_{II} phase crystallites [16]. It is possible that the crystallites observed in electron microscopy may be obtained under rather special conditions, not typical of those in our pressure-jump cells. However, until this question is settled, sequential models of relaxation are not consistent with all that is known about the H_{II} phase.

The most interesting question concerns the microscopic origin of the energy barriers involved in the nonexponential kinetics, and an understanding of the processes determining the time scales involved in the transport of water within and into liquid crystalline domains. Studies on single crystals would be useful in that the domain size effects can be sorted out, but it is difficult to grow single crystals of the H_{II} phase. Studies of transport in different liquid crystalline topologies, such as the lamellar or cubic phases, may also provide insight into the microscopic origin of the energy barriers. Whatever the microscopic mechanism, the fact that the pores are formed by elastic membranes may be expected to give rise to nonlinear effects rather different from conventional studies on fluid invasion into porous media, where the substrate forms a rigid framework.

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