

Highly Aligned Lipid Membrane Systems in the Physiologically Relevant "Excess Water" Condition

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ABSTRACT The "excess water" condition in biologically relevant systems is met when a membrane mesophase coexists with excess bulk water. Further addition of water to such a system results in no change to any of the system's physical properties (e.g., transition temperature, repeat spacing, and structural mesophases). Moreover, because biological membranes are anisotropic systems, many of their properties are best studied using aligned samples. Although model membrane systems are routinely aligned, they have traditionally been hydrated with water vapor. It is well known that membranes exposed to water vapor at 100% humidity do not imbibe the same quantity of water as a sample in contact with liquid water. As such, membranes that have been hydrated with water vapor have physical properties different from those of membranes dispersed in water. Because of this shortcoming, aligned membranes have not been utilized to their full potential. Here we present a novel and simple method of aligning model membrane systems under conditions of excess water, which will make possible, for the first time, a variety of techniques (e.g., neutron and x-ray diffraction, nuclear magnetic resonance, electron spin resonance, attenuated total reflection infrared spectroscopy, etc.) for studying such systems under physiologically relevant conditions. In addition, when dealing with samples of limited availability, the system allows for the conditions (buffer pH and ionic strength) to be altered without any effect on the sample's alignment.

INTRODUCTION

Highly aligned membrane systems, when compared to powder samples or commonly referred to liposomes, have made possible a variety of techniques for extracting unambiguous structural information from a number of model membranes (e.g., Fringeli, 1977; Kar et al., 1985; Sirota et al., 1988; Raghunathan and Katsaras, 1995; Prosser et al., 1996), and in some cases have led to structural solutions only when an aligned sample became available (Smith et al., 1988; Sirota et al., 1988; Raghunathan and Katsaras, 1995; Katsaras et al., 1995). When diffraction is the technique of choice, aligned samples also allow for clear differentiation between in-plane and out-of-plane correlations (Smith et al., 1988; Raghunathan and Katsaras, 1995). Because of the fact that the signal is not isotropic, as is the case for powders, data collection is accelerated, and this reduction in acquisition times holds true for practically all of the techniques used to characterize such systems. In addition, the amount of material required to make aligned samples is generally a fraction of that needed for liposomal preparations. The use of aligned samples is highly desirable; however, their major drawback, until now, has been that they could not be produced under "biologically relevant" conditions, which are generally accepted as being the following: 1) The lipids, which form a bilayer, are in the liquid crystalline L_{α} phase,

in which no long-range order within the 2D layers exists as a result of the rapid translational diffusion and *trans-gauche* isomerizations of the fatty acid chains. 2) The membranes are placed in an excess of water such that at all times there is a coexistence of lipid/water and water phases. 3) The aqueous environment reflects relevant physiological pH and ionic strength conditions.

Although lipid bilayers and membranes have been aligned by a variety of methods (e.g., Clark et al., 1980; Smith et al., 1988; Prosser et al., 1996; Katsaras et al., 1997), the most commonly used, and possibly the most practical, is the alignment of a membrane on a solid substrate of either glass (Torbet and Wilkins, 1976; Raghunathan and Katsaras, 1995) or silicon (Katsaras and Jeffrey, 1997) from a concentrated lipid/solvent solution. This well-known method of sample preparation results in several thousand highly aligned multibilayers with a mosaic spread of only a few degrees when the samples are hydrated in a 100% relative humidity (RH) environment. However, when compared to liposomes in contact with water, aligned samples hydrated with water vapor have a reduced level of hydration (Jendrasiak and Hasty, 1974). This so-called vapor pressure paradox is widely recognized and results in the multibilayers having reduced repeat or *d*-spacings (Torbet and Wilkins, 1976), elevated transition temperatures, and different mesophases (Chapman et al., 1967; Sackmann, 1983). Attempts to immerse aligned multibilayers in water result in the destruction of the orientation of the lipids (Morrison, 1993).

In the late 1970s, Powers and Pershan (1977) managed to align dipalmitoylphosphatidylcholine (DPPC) multibilayers up to a water concentration of 30 wt%, after which the samples exhibited a visible deterioration in alignment. At

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~30 wt% water there is a coexistence of hydrated $L_{\beta'}$ DPPC multibilayers with bulk water. However, it is only at concentrations of water of ~38 wt% or greater that the excess water condition is met for the biologically relevant L_{α} phase. Although the transition temperatures for the various mesophases are not affected by water concentrations being slightly less than excess, structural parameters such as repeat spacings are altered (Janiak et al., 1976; Ruocco and Shipley, 1982). Powers and Pershan (1977) determined the macroscopic orientation of the samples by using microscopy and conoscopy, but no mention was made of the degree of alignment of the DPPC multibilayers (Powers and Pershan, 1977). Although sample alignment was maintained with decreasing temperature, on heating, "alignment was often destroyed" (Powers and Pershan, 1977). Finally, in the last few years, there have been two studies that have produced fully hydrated $L_{\beta'}$ phase DPPC multibilayers by allowing water to condense on an aligned sample (Katsaras et al., 1992; Tristram-Nagle et al., 1993).

The transition temperatures for the various mesophases of DPPC and dihexadecylphosphatidylethanolamine (DHPE) liposomes in excess water, along with their corresponding d -spacings, have been accurately determined by a variety of methods (e.g., Janiak et al., 1976; Torbet and Wilkins, 1976; Chen et al., 1980; Ruocco and Shipley, 1982; Stümpel et al., 1983; Caffrey, 1985; Katsaras et al., 1986; Hing et al., 1991; Mencke and Caffrey, 1991; Zhang et al., 1996; Nagle et al., 1996). Using a novel and simple method of aligning model membrane systems (Fig. 1), we have studied aligned multibilayers of DPPC and DHPE immersed in water by using neutron diffraction. The results show that the samples are highly aligned ($\leq 0.5^\circ$ mosaic spread) and that there is an excellent agreement in both transition temperatures and d -spacings for the various mesophases (e.g., L_{β} , $L_{\beta'}$, $P_{\beta'}$, L_{α} , and H_{II}) between the aligned samples in excess water and the liposomal preparations in excess water. All of the samples proved to be stable over many days and sometimes weeks of experimentation, with no change in the scattering intensity for the various mesophases.

MATERIALS AND METHODS

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-Di-O-hexadecyl-*sn*-glycero-3-phosphoethanolamine (DHPE) were purchased from Avanti Polar Lipids (Birmingham, AL) and used without any further purification. Deuterated water (D_2O), with a purity of 99.95% and a pH of 6.4, was kindly supplied by Atomic Energy of Canada (Chalk River, Ontario, Canada).

Aligned bilayers were prepared as follows: 0.5 ml of a concentrated lipid/methanol solution (40 mg/ml) was pipetted onto the highly polished surface of a silicon wafer of dimensions 1 cm \times 5 cm. After the evaporation of the methanol, a clear film was left adhering to the surface of the silicon substrate. The remainder of the methanol was then evaporated by placing the samples under a vacuum for 12 h, after which two teflon spacers (1 cm \times 0.5 cm \times 0.005 cm) and an additional 1 cm \times 5 cm silicon wafer were placed on top of the sample, creating a silicon/lipid/teflon/silicon "sandwich" (Fig. 1). The two teflon spacers, located at either end of the rectangular silicon wafers, are an integral part of the system, maintaining a gap of ~0.005 cm between the two wafers. In arriving at the

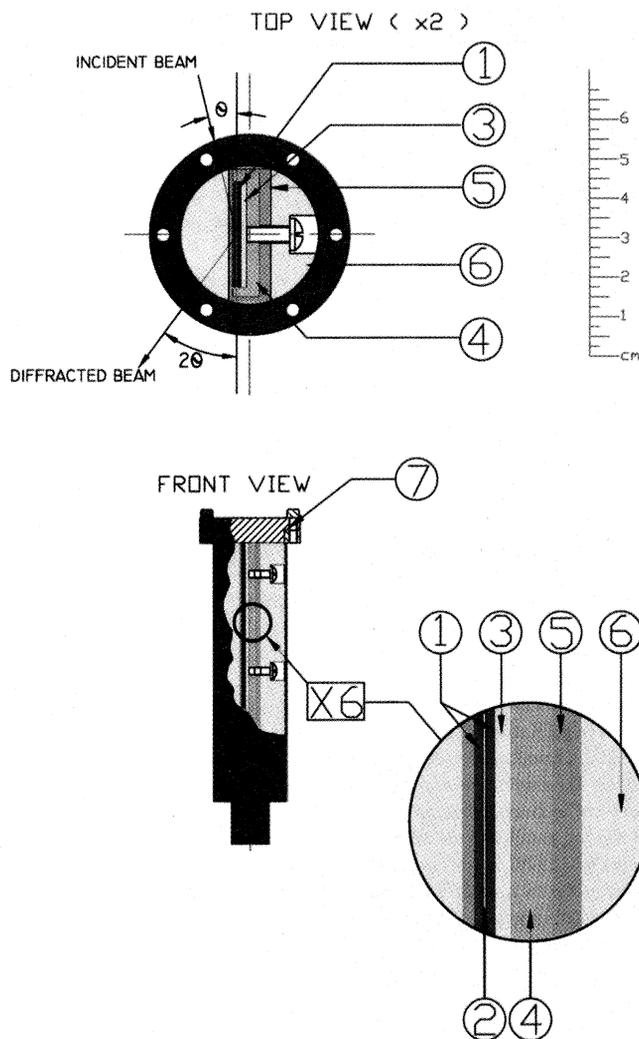


FIGURE 1 Detailed drawing of the sample holder and diffraction geometry used for the present experiments. (1) Silicon/teflon assembly containing the aligned lipid multibilayers. (2) Lipid multibilayers. (3) Aluminum pressure plate used to maintain 0.005 cm gap. (4) Water reservoir. (5) Removable aluminum cassette that retains the silicon/teflon assembly and the pressure plate. (6) Aluminum billets used to exclude water. (7) Indium seal. The choice of materials used to construct the sample holder was made simply on the basis that both aluminum and silicon are "transparent" to neutrons. The idea behind the "cassette assembly" (4) will hold true for all other techniques designed to make use of aligned membranes in excess water conditions, the only difference being that suitable materials must be chosen. Of further significance is that the conditions of the water reservoir (e.g., pH, ionic strength) can be altered without affecting the alignment of the sample. This is done simply by emptying the reservoir and refilling it with the appropriate solution.

thickness of the teflon spacers, no systematic tests were performed. As such, it is possible that slightly different thicknesses for the spacer can be used, with similar results. The sandwich, along with an aluminum pressure plate, was then placed and secured in the specimen "cassette" by two screws located toward either end of the cassette (Fig. 1). At this point, the cassette is introduced into the sample holder and the water reservoir filled with D_2O . The sample is now completely immersed in water, allowing the various mesophases to draw upon the water reservoir as required, to achieve their limiting repeat spacing and transition temperature values. In the aligned sample, the bilayer normal is orthogonal to the silicon substrate.

Using this method of preparing aligned, fully hydrated bilayers, it was found that the alignment of the multibilayer stacks ($\leq 0.5^\circ$ mosaic) was maintained on both increasing and decreasing temperatures.

An aluminum thermal shield placed over the sample holder was used to minimize thermal gradients in the sample while the temperature was controlled by a water bath, the water of which was circulated through the base of the sample holder. It should be noted that although we used silicon to align the DPPC and DHPE multibilayers, other materials such as glass and a variety of crystalline materials (e.g., germanium wafers) can be used successfully as a substitute for the silicon wafers.

The experiments were carried out at the Chalk River Laboratories NRU reactor, using the N5 triple-axis spectrometer, which has a neutron flux of $\sim 5.4 \pm 0.3 \times 10^9$ neutrons $\text{cm}^{-2} \text{s}^{-1}$ at the monochromator position. Monoenergetic neutrons with a wavelength of $2.37 \text{ \AA} \pm 0.005 \text{ \AA}$ were selected by using the (002) reflection of a pyrolytic-graphite monochromator (mosaic $\approx 0.4^\circ$), and higher order neutrons were eliminated with a graphite filter. The wavelength was determined by using a powder sample of aluminum. The instrument resolution for the N5 spectrometer was defined by Soller collimators, yielding an overall calculated ΔQ (FWHM) of 0.008 \AA^{-1} .

The Bragg maxima in Figs. 2 and 3 were recorded using θ - 2θ or radial scans. Because the scattering angle is twice the incident angle, every time the sample is rotated by an angle θ , the detector is rotated by an angle 2θ (Fig. 1). Rocking curves (Figs. 2 and 3, insets) are a measure of the angular distribution of the lipid multibilayers with respect to the substrate and are commonly obtained by fixing the detector on a Bragg peak ($2\theta_B$) and rotating the sample through a series of angles ψ with respect to the incident beam (e.g., Katsaras et al., 1997; Miceli and Palmström, 1995; Gibaud et al., 1993; Franks and Lieb, 1979; Büldt et al., 1979; Worcester and Franks, 1976). The minima seen in the rocking curves (Figs. 2 and 3, insets) are the result of increased absorption when either the incident or diffracted beam is aligned parallel to the silicon substrate as the sample is rotated (Franks and Lieb, 1979; Büldt et al., 1979). It should be noted that both θ and ψ refer to the rotation of the sample and are used to differentiate between a θ - 2θ scan (Figs. 2 and 3) and rocking scans (Figs. 2 and 3, insets), respectively.

RESULTS AND DISCUSSION

In the present experiments diffraction was used, as it is the least ambiguous method of structural determination, whereas neutrons are an ideal probe when it comes to complicated sample environments, as many materials (e.g., aluminum) are transparent to them. In the case of neutrons, the incoherent scattering and absorption cross sections for both aluminum and silicon are small compared to their coherent scattering cross sections (Sears, 1992). As such, the sample assembly was constructed entirely from aluminum (Fig. 1). As a result of having an aligned sample, the scattering does not occur isotropically, as is the case in liposomal preparations, samples of which are effectively powders. The advantage of nonisotropic scattering is twofold: 1) faster data collection, as the signal is not spread over 2π ; 2) clear differentiation between in-plane and out-of-plane structure (Smith et al., 1988; Raghunathan and Katsaras, 1995). D_2O instead of H_2O was used, because the incoherent cross section of deuterium for neutrons is small compared to that for hydrogen (Sears, 1992). Because the sample is predominantly composed of water, the use of D_2O in place of H_2O results in a better signal-to-noise ratio, which in real terms translates into a faster rate of data collection.

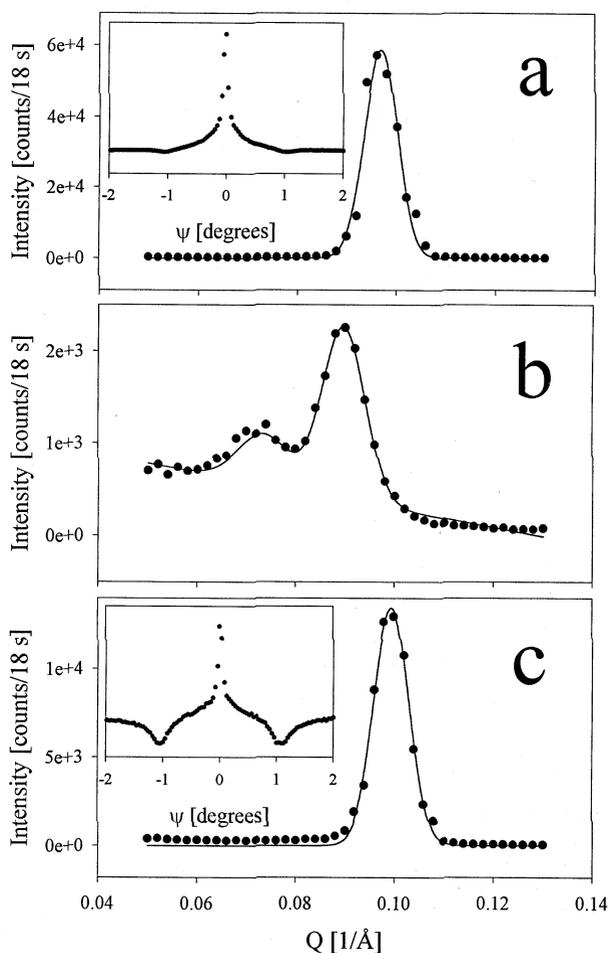


FIGURE 2 Resolution-limited Bragg maxima of highly aligned ($\leq 0.5^\circ$ mosaic) DPPC multibilayers in the (a) L_α phase at 49.2°C with a lamellar d -spacing of 65.4 \AA ; (b) 70.5-\AA multibilayers in the P_β phase at a temperature of 40.5°C ; and (c) L_β' multibilayers with a d -spacing of 63.5 \AA at a temperature of 19.9°C . The error in the d -spacing measurements is $\pm 0.3 \text{ \AA}$, and for the temperature measurement the error is $\pm 0.5^\circ\text{C}$. Bragg maxima were fitted using a single Gaussian function, except in the case of the P_β phase, where two such functions were needed to fit the pattern. The rocking curves or transverse scans (insets) for the first-order Bragg peak show that the sample is highly aligned, exhibiting a mosaic of $\leq 0.5^\circ$.

The most widely studied model membrane system, DPPC/water, has been characterized over the years by numerous studies (e.g., Chapman et al., 1967; Jendrasiak and Hasty, 1974; Torbet and Wilkins, 1976; Fringeli, 1977; Sackmann, 1983; Katsaras, 1995; Raghunathan and Katsaras, 1995); its phase diagram was first established in the 1960s (Chapman et al., 1967). In the excess water condition, the gel-to-liquid crystalline phase transition occurs at $\sim 41.5^\circ\text{C}$. However, when the sample contains only 5 wt% water, the transition temperature increases to 75°C (Sackmann, 1983). Under conditions of excess water, DPPC liposomes have been shown by x-ray diffraction to have d -spacings between 60 \AA (Janiak et al., 1976) and 67.2 \AA (Zhang et al., 1996; Nagle et al., 1996) in the liquid crystalline L_α phase, whereas for the L_β' and P_β' phases, d -spacings of $\sim 63.5 \text{ \AA}$ (Torbet and Wilkins, 1976; Stümpel et

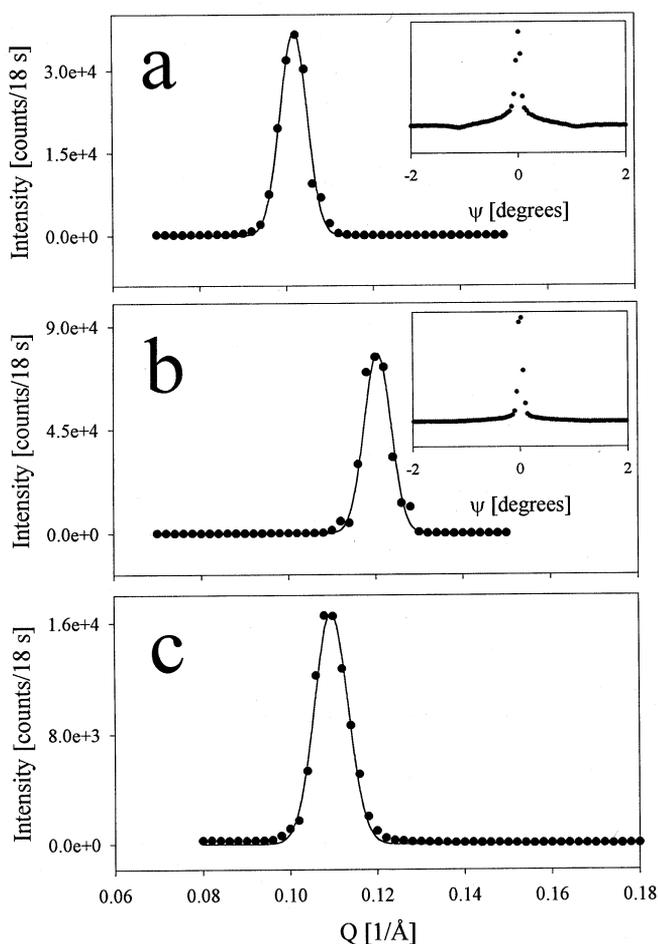


FIGURE 3 Bragg maxima of aligned DHPE multibilayers in the $L_{\beta'}$ (a), L_{α} (b), and H_{II} (c) phases, with d -spacings of 62.2 Å (20°C), 52.4 Å (69.6°C), and 57.6 Å (91.9°C), respectively. The insets shown in a and b show that the samples are highly aligned, with rocking curves of $\leq 0.5^\circ$ for the first-order Bragg peak. Except for the H_{II} phase (c), Bragg maxima of the lamellar phases (a and b) are resolution limited.

al., 1983; Katsaras et al., 1986; Nagle et al., 1996) and ~ 70 Å (Janiak et al., 1976; Stamatoff et al., 1982; Stümpel et al., 1983), respectively, are commonly reported.

Fig. 2 depicts the first-order lamellar Bragg maxima for three phases of DPPC, obtained using an aligned multilamellar sample in excess water (Fig. 1). At 49.2°C, L_{α} multibilayers exhibit a d -spacing of 65.4 Å ($Q = 0.096$ Å $^{-1}$) (Fig. 2 a), in good agreement with values found in the literature for DPPC liposomes in excess water (Zhang et al., 1996; Nagle et al., 1996). It should be noted that d values for this phase are known to vary noticeably (Janiak et al., 1976; Stamatoff et al., 1982; Stümpel et al., 1983; Wolfe et al., 1992; Zhang et al., 1996), even from sample to sample within the same experiment (Zhang et al., 1996). The rocking curve for the aligned L_{α} DPPC multibilayers is shown in the inset of Fig. 2 a; its shape is typical of epitaxially grown samples (Gibaud et al., 1993; Miceli and Palmström, 1995). As can be seen, the samples are extremely well aligned, having a mosaic of $\leq 0.5^\circ$. On decreasing the temperature, a

pure $P_{\beta'}$ phase was obtained at 40.5°C, in which the multibilayers had a d -spacing of 70.5 Å and where the [1,0] reflection of the oblique unit cell (Janiak et al., 1976) is centered at a Q value of 0.089 Å $^{-1}$ (Fig. 2 b). Both the temperature for the $L_{\alpha} \rightarrow P_{\beta'}$ transition (Janiak et al., 1976; Ruocco et al., 1982; Katsaras et al., 1986) and the repeat spacing (Stamatoff et al., 1982; Ruocco and Shipley, 1982; Stümpel et al., 1983) are in agreement with the values found in the literature. The peak at $Q = 0.073$ Å $^{-1}$ is a result of the [1,1] reflection of the oblique unit cell. Upon further reduction of the temperature, a pure $L_{\beta'}$ phase was obtained at 31.4°C, again in agreement with previously reported data (Janiak et al., 1976; Stümpel et al., 1983; Katsaras et al., 1986). Fig. 2 c shows the first-order Bragg diffraction maximum corresponding to the widely reported repeat spacing of 63.5 Å ($Q = 0.099$ Å $^{-1}$) for DPPC liposomes in the $L_{\beta'}$ phase at a temperature of $\sim 20^\circ$ C (Stamatoff et al., 1982; McIntosh and Simon, 1986; Nagle et al., 1996). As was the case for DPPC multibilayers in the L_{α} phase, the rocking curve (Fig. 2 c, inset) for the $L_{\beta'}$ multibilayers demonstrates that the system is again highly aligned ($\leq 0.5^\circ$ mosaic). As mentioned previously, when compared to samples in the excess water condition, 100% RH aligned DPPC multibilayers also exhibit repeat distances, which are much smaller for all mesophases (Alecio et al., 1985; Sirota et al., 1988; Katsaras, 1995; Katsaras and Jeffrey, 1997).

Glycerophospholipids with choline (e.g., DPPC) and ethanolamine polar headgroups are the main constituents of plasma and subcellular membranes. As such, to further demonstrate the effectiveness of the technique on the other major group of phospholipids, experiments similar to those described for DPPC/water were carried out with the lipid DHPE. Dispersions of this lipid have been characterized extensively with both calorimetric (Hing et al., 1991) and diffraction (Hing et al., 1991; Caffrey, 1985; Mencke and Caffrey, 1991) measurements. Liposomal preparations of this membrane system, under conditions of excess water, have been shown to undergo two thermotropic phase transitions occurring at 67°C ($L_{\beta} \rightarrow L_{\alpha}$) and 92°C ($L_{\alpha} \rightarrow H_{II}$ (inverted hexagonal)) (Hing et al., 1991; Caffrey, 1985; Mencke and Caffrey, 1991).

Fig. 3 a shows the first-order Bragg maximum of the aligned DHPE/water system in the L_{β} phase, corresponding to a d -spacing of 62.2 Å ($Q = 0.101$ Å $^{-1}$) at a temperature of 20°C, in agreement with the x-ray diffraction data of DHPE/water liposomes at 22°C, with a d -spacing of 62.5 Å (Hing et al., 1991). Increasing the temperature causes the system to undergo the well-known $L_{\beta} \rightarrow L_{\alpha}$ phase transition at $\sim 68^\circ$ C. Fig. 3 b shows the first-order Bragg maximum of the pure L_{α} phase at 69.6°C, where the aligned excess water bilayers have a d -space value of 52.4 Å ($Q = 0.120$ Å $^{-1}$) (Caffrey, 1985; Mencke and Caffrey, 1991). Further increases in temperature resulted in the L_{α} phase transforming into the inverted hexagonal phase at a temperature of $\sim 90^\circ$ C. Fig. 3 c shows the [1,0] reflection of the two-dimensional hexagonal lattice, which corresponds to a repeat spacing of 57.6 Å ($Q = 0.109$ Å $^{-1}$) and is in line with

x-ray data of DHPE liposomes in excess water (Caffrey, 1985; Mencke and Caffrey, 1991). Of note is the fact that from 0 to 10 wt% H₂O, DHPE liposomes enter the H_{II} phase directly from the L_β phase, bypassing altogether the L_α phase (Hing et al., 1991). Only above 15 wt% water are all three phases observed, and only above 19 wt% do the transition temperatures and *d*-spacings attain their limiting values (Hing et al., 1991).

The results in this paper are seemingly in contradiction with a recent theoretical paper by Podgornik and Parsegian (1997), where they predict that for systems adsorbed to a solid substrate, the bilayer undulations (which partly contribute to the *d*-spacing of the system) are suppressed. This dampening effect of the bilayer's mechanical undulations manifests itself in the reduced *d*-spacing of the multibilayers, especially those bilayers in the biologically relevant L_α phase. This indeed would be the case if the bilayers were not in contact with liquid water but with water vapor! However, in the present experiments the 0.005-cm teflon spacers allow for the direct contact of liquid water on one side of the sample, which then allows the bilayers to undulate. Once this occurs, the energy costs for the other side of the sample to "lift off" from the substrate to allow undulation are negligible. As such, the silicon substrates act in "confining" the sample. Without the use of the teflon spacers, the maximum *d*-spacing obtained for L_β and L_α phase DPPC multibilayers was 62.8 Å and 61.6 Å, respectively.

SUMMARY AND CONCLUDING REMARKS

It has been stated that the best way to ensure full hydration (excess water) in aligned membrane systems is to have the samples immersed in water (Morrison, 1993). "Unfortunately the orientation of the lipids (DPPC) cannot be maintained under these conditions" (Morrison, 1993). Here we have reported on two membrane systems (DPPC/water and DHPE/water) composed of lipids with the two most common types of polar headgroups, which have been aligned under conditions in which full hydration for all mesophases is ensured. In addition, emptying the water reservoir allows for new conditions of pH and ionic strength to be introduced without any disruption to the alignment of the sample, an important consideration when dealing with samples of limited availability. This novel method now opens up the path for the study of highly aligned, biologically relevant membrane systems by a variety of techniques (e.g., x-ray and neutron diffraction, nuclear magnetic resonance, electron spin resonance, attenuated total reflection infrared spectroscopy, etc.) under physiologically meaningful conditions.

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REFERENCES

- Alecio, M. R., A. Miller, and A. Watts. 1985. Diffraction of x-rays by rippled phosphatidylcholine bilayers. *Biochim. Biophys. Acta.* 815: 139–142.
- Büldt, G., H. U. Gally, J. Seelig, and G. Zaccari. 1979. Neutron diffraction studies on phosphatidylcholine model membranes. *J. Mol. Biol.* 134: 673–691.
- Caffrey, M. 1985. Kinetics and mechanism of the lamellar gel/lamellar liquid-crystal and lamellar/inverted hexagonal phase transition in phosphatidylethanolamine: a real-time x-ray diffraction study using synchrotron radiation. *Biochemistry.* 24:4826–4844.
- Chapman, D., R. M. Williams, and B. D. Ladbroke. 1967. Physical studies of phospholipids. VI. Thermotropic and lyotropic mesomorphism of some 1,2-diacylphosphatidylcholines (lecithins). *Chem. Phys. Lipids.* 1:445–475.
- Chen, S. C., J. M. Sturtevant, and B. J. Gaffney. 1980. Scanning calorimetric evidence for a third phase transition in phosphatidylcholine bilayers. *Proc. Natl. Acad. Sci. USA.* 77:5060–5063.
- Clark, N. A., K. J. Rothschild, D. A. Luippold, and B. A. Simon. 1980. Surface-induced lamellar orientation of multilayer membrane arrays: theoretical analysis and a new method with application to purple membrane fragments. *Biophys. J.* 31:65–96.
- Franks, N. P., and W. R. Lieb. 1979. The structure of lipid bilayers and the effects of general anaesthetics: an x-ray and neutron diffraction study. *J. Mol. Biol.* 133:469–500.
- Fringeli, U. P. 1977. The structure of lipids and proteins studied by attenuated total reflection (ATR) infrared spectroscopy. *Z. Naturforsch.* 32c:20–45.
- Gibaud, A., R. A. Cowley, D. F. McMorro, R. C. C. Ward, and M. R. Wells. 1993. High-resolution x-ray scattering study of the structure of niobium thin films on sapphire. *Phys. Rev. B.* 48:14463–14471.
- Hing, F. S., P. R. Maulik, and G. G. Shipley. 1991. Structure and interactions of ether- and ester-linked phosphatidylethanolamines. *Biochemistry.* 30:9007–9015.
- Janiak, M., D. M. Small, and G. G. Shipley. 1976. Nature of the thermal pretransition of synthetic phospholipids: dimyristoyl- and dipalmitoyl- lecithin. *Biochemistry.* 15:4575–4580.
- Jendrasiak, G. L., and J. H. Hasty. 1974. The hydration of phospholipids. *Biochim. Biophys. Acta.* 337:79–91.
- Kar, L., E. Ney-Igner, and J. H. Freed. 1985. Electron spin resonance and electron-spin-echo study of oriented multilayers of L_α dipalmitoylphosphatidylcholine water system. *Biophys. J.* 48:569–595.
- Katsaras, J. 1995. Structure of the subgel (L_c) and gel (L_β) phase of oriented dipalmitoylphosphatidylcholine multibilayers. *J. Phys. Chem.* 99:4141–4147.
- Katsaras, J., R. L. Donaberger, I. P. Swainson, D. C. Tennant, Z. Tun, R. R. Vold, and R. S. Prosser. 1997. Rarely observed phase transitions in a novel lyotropic liquid crystal system. *Phys. Rev. Lett.* 78:899–902.
- Katsaras, J., and K. R. Jeffrey. 1997. Evidence of the hydration force in gel phase lipid multibilayers. *Europhys. Lett.* 38:43–48.
- Katsaras, J., V. A. Raghunathan, E. J. Dufourc, and J. Dufourcq. 1995. Evidence for a two-dimensional molecular lattice in the subgel phase of DPPC bilayers. *Biochemistry.* 34:4684–4688.
- Katsaras, J., R. H. Stinson, E. J. Kendall, and B. D. McKersie. 1986. Structural simulation of free radical damage in a model membrane system: a small-angle x-ray diffraction study. *Biochim. Biophys. Acta.* 861:243–250.
- Katsaras, J., D. S.-C. Yang, and R. M. Epan. 1992. Fatty-acid chain tilt angles and directions in dipalmitoyl phosphatidylcholine bilayers. *Biophys. J.* 63:1170–1175.
- McIntosh, T. J., and S. A. Simon. 1986. Hydration force and bilayer deformation: a reevaluation. *Biochemistry.* 25:4058–4066.
- Mencke, A. P., and M. Caffrey. 1991. Kinetics and mechanism of the pressure-induced lamellar order/disorder transition in phosphatidylethanolamine: a time-resolved x-ray diffraction study. *Biochemistry.* 30:2453–2463.
- Miceli, P. F., and C. J. Palmström. 1995. X-ray scattering from rotational disorder in epitaxial films: an unconventional mosaic crystal. *Phys. Rev. B.* 51:5506–5509.

- Morrison, C. 1993. Polyethylene glycol as a hydration agent in oriented membrane bilayer samples. *Biophys. J.* 64:1063–1068.
- Nagle, J. F., R. Zhang, S. Tristram-Nagle, W. Sun, H. I. Petrache, and R. M. Suter, R. M. 1996. X-ray structure determination of fully hydrated L_{α} phase dipalmitoylphosphatidylcholine bilayers. *Biophys. J.* 70:1419–1431.
- Podgornik, R., and V. A. Parsegian. 1997. On a possible microscopic mechanism underlying the vapor pressure paradox. *Biophys. J.* 72:942–952.
- Powers, L., and P. S. Pershan. 1977. Monodomain samples of dipalmitoyl phosphatidylcholine with varying concentrations of water and other ingredients. *Biophys. J.* 20:137–152.
- Prosser, R. S., S. A. Hunt, J. A. DiNatale, and R. R. Vold. 1996. Magnetically aligned membrane model systems with positive order parameter: switching the sign of the S_{zz} with paramagnetic ions. *J. Am. Chem. Soc.* 118:269–270.
- Raghunathan, V. A., and J. Katsaras. 1995. Structure of the L_{α} phase in a hydrated lipid multilamellar system. *Phys. Rev. Lett.* 74:4456–4459.
- Ruocco, M. J., and G. G. Shipley. 1982. Characterization of the subtransition of hydrated dipalmitoylphosphatidylcholine bilayers. *Biochim. Biophys. Acta.* 684:59–66.
- Sackmann, E. 1983. Physical foundations of the molecular organization and dynamics of membranes. In *Biophysics*. W. Hoppe, editor. Springer Verlag, New York. 425–457.
- Sears, V. F. 1992. Neutron scattering lengths and cross sections. *Neutron News.* 3:26–37.
- Sirota, E. B., G. S. Smith, C. R. Safinya, R. J. Plano, and N. A. Clark. 1988. X-ray scattering studies of aligned, stacked surfactant membranes *Science.* 242:1406–1409.
- Smith, G. S., E. B. Sirota, C. R. Safinya, and N. A. Clark. 1988. Structure of the L_{β} phase in a hydrated phosphatidylcholine multimembrane. *Phys. Rev. Lett.* 60:813–816.
- Stamatoff, J., B. Feuer, H. J. Guggenheim, G. Tellez, and T. Yamane. 1982. Amplitude of rippling in the P_{β} phase of dipalmitoylphosphatidylcholine bilayers. *Biophys. J.* 38:217–226.
- Stümpel, J., H. Eibl, and A. Nicksch. 1983. X-ray analysis and calorimetry on phosphatidylcholine model membranes: the influence of length and position of acyl chains upon structure and phase behavior. *Biochim. Biophys. Acta.* 727:246–254.
- Torbet, J., and M. H. F. Wilkins. 1976. X-ray diffraction studies of lecithin bilayers. *J. Theor. Biol.* 62:447–458.
- Tristram-Nagle, S., R. Zhang, R. M. Suter, C. R. Worthington, W.-J. Sun, and J. F. Nagle. 1993. Measurement of chain tilt angle in fully hydrated bilayers of gel phase lecithins. *Biophys. J.* 64:1097–1109.
- Wolfe, D. H., L. J. Lis, O. Kucuk, M. P. Westerman, B. A. Cunningham, B. A. S. B. Qadri, W. Bras, and P. J. Quinn. 1992. Phase transitions between ripple structures in hydrated phosphatidylcholine-cholesterol multilamellar assemblies. *Phys. Rev. Lett.* 68:1085–1088.
- Worcester, D. L., and N. P. Franks. 1976. Structural analysis of hydrated egg lecithin and cholesterol bilayers. II. Neutron diffraction. *J. Mol. Biol.* 100:359–378.
- Zhang, R., S. Tristram-Nagle, W. Sun, R. L. Headrick, T. C. Irving, R. M. Suter, and J. F. Nagle. 1996. Small-angle x-ray scattering from lipid bilayers is well described by modified Caillé theory but not by paracrystalline theory. *Biophys. J.* 70:349–357.