X-ray Scattering Studies of Aligned, Stacked Surfactant Membranes

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darkness alone could have brought about the Cretaceous-Tertiary boundary extinction event only if its duration was greater than 3 to 5 months or if the dinosaurs were occupying high latitudes only during part of the year, which may be unlikely for some of the small, large-eyed, large-brained Victorian hypsilophodontids.

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32. We thank Atlas Copco, the National Geographic Society, the Australian Research Grants Scheme, David Holdings Ltd., Mobil Oil, Inland Chemical Industries, J. Herman, W. Load, J. Chessells, the Friends and Council of the Museum of Victoria, and the Sunshine Foundation for supporting this research. L. Kool for preparing much of the material; and D. Gelt, S. Morton, F. Coffa, P. Hermansen, and S. Fay for graphics, photography, and manuscript preparation. The isotopic analyses were made possible through the support from the Australian Research Council, National Energy Research, Development and Demonstration Council, and Esso Australia. I. Duddy kindly allowed access to his extensive concretion collection and his sedimentological analysis of the Otway Group.

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X-ray scattering studies were performed to understand the structure and correlations in the lamellar phases of thick, freely suspended films of (i) the hydrated phospholipid dimyristoylphosphatidylcholine (DMPC) and (ii) the ternary system consisting of the surfactant sodium dodecyl sulfate (SDS), cosurfactant (pentanol), and water. The films were drawn in a temperature- and humidity-controlled environment, where the layers were oriented to within 0.1°. In the DMPC system, this made it possible to directly observe the orientation of the $P_2$ modulation and to identify phase $L_0^*$ as three distinct phases distinguished by the direction of chain tilt with respect to the lattice. In the $L_0^*$ phase of the ternary system, power law behavior of the (0,1,1) structure factor arising from the algebraic decay of layer correlations was observed in single crystals.

Since the lipid mosaic model of cell membranes was proposed (1), much work has been done to understand the details of their function and structure. Although actual cell membranes consist of many components including lipids, cholesterol, and proteins, a great deal can be learned from the study of simple model systems that contain either a subset of those components or components similar to those found in real membranes. These are lyotropic, liquid crystalline phases (2-5), which are also of scientific interest because they are prototype models for elucidating the nature of phases and their transitions in two-dimensional (2D) systems. Furthermore, because of their ability to buckle out of their 2D plane, these phases provide real models for studies of the statistical physics of crumpled surfaces imbedded in three-dimensional (3D) space (6).

The high-water-content portion of the phase diagram of phospholipids was known to consist of three lamellar regions. The $L_0^*$ phase, characterized by disordered chains and a lack of positional order within the layer, has been the subject of a great deal of study (2-5) because surfactants in most living membranes are in this state. The $P_2$ phase is of particular interest as a result of the presence of a long wavelength (80 Å < $\lambda$ < 120 Å) modulation of the layers. The $L_0^*$ phase was believed to have tilted molecules, and although occupying a large portion of the phase diagram, it was largely ignored. We have expanded on a technique (7) by which we can produce extremely well-aligned, freely suspended films of the samples in continuously variable humidity conditions.

![Fig. 1. The temperature-humidity phase diagram of DMPC. Because both axes are related to thermodynamic potentials, there are no two-phase regions. Note that the phase previously known as $L_0^*$ is, in fact, three distinct phases, $L_0^*$, $L_{22}$, and $L_{33}$.](image-url)
The utility in studying thennotropic study of biologically relevant systems. In this report, we show its importance in the alignment. This technique has been of great utility in studying thermotropic (dry) liquid crystals and the physics of 2D systems (7-9).

In this report, we show its importance in the study of biologically relevant systems.

The phospholipid chosen for study was DMPC (10). We were able to produce freely suspended films of hydrated lipids in a controlled humidity environment (2400 bilayers thick). By smearing hydrated Lα sample across a hole (5 mm in diameter) in a thin plate, the lamellae in the film oriented to within 0.1° of the surface normal. Because these systems often form weakly ordered structures where only a few scattering peaks are observed, such a high degree of orientation is essential for determining many structural properties of these phases. The films were drawn and the measurements performed in a chamber where the temperature (T) was controlled by means of thermoelectric devices and the relative humidity (RH) was controlled to ±0.2% by means of a gas flow system (5) with dew point-sensing hygrometers for feedback and reading. By varying the humidity, which is directly related to the chemical potential of water [Δμ = RT ln(RH/100), where R is the gas constant], we can continuously vary the amount of water in the layers.

The phase diagram (Fig. 1) was mapped out reproducibly as a function of T and RH. Because RH is effectively a thermodynamic potential, there are no two-phase regions in this phase diagram, such as one obtains with gravitometrically prepared samples (4). The films in these three phases have characteristic textures when viewed with reflected white light (Fig. 2), which helped in mapping out the phase diagram. The Lα phase appears very smooth and shiny. The three Lβ phases, indistinguishable optically, appeared rougher. The Pβ phase appears grainy in texture.

The scattering studies were done on Huber four-circle diffractometers with an 18-kW Rigaku rotating anode x-ray generator at Exxon, and also with synchrotron radiation at the Stanford Synchrotron Radiation Laboratory beam-line 7-2 and the National Synchrotron Light Source (Brookhaven) Exxon beam-line X10A. The sample cell was designed to allow both scattering in transmission to study the in-plane structure and reflection from the surface of the film to measure the bilayer repeat distance (d) as well as the nature and magnitude of layer undulations.

We were able to directly observe in the Pβ phase the orientation of the modulation wave vector, on a freely suspended aligned sample. The sample was about 2 μm thick (~400 bilayers), and the layer normals were aligned to better than 1°. The modulation, measured at T = 20.6°C and 94% RH where d = 55.9 Å, was shown to have a wave vector of magnitude q = 0.035 ± 0.002 Å⁻¹ (λ = 180 Å) tilted 7.5° out of the plane of the layers. This is consistent with interpretations of powder diffraction data (11). Figure 3A shows a scan in reciprocal space along the direction of the modulation where four orders of reflections are visible. Because of the orientational quality of our samples and the use of a high-resolution x-ray spectrometer at the synchrotron facilities, we were able to obtain unique information on the structural nature of the modulation. The anharmonic nature of the modulation is evident, consistent with freeze-fracture direct image data (12), by the appearance of higher harmonics of the modulation.

Figure 3B shows a q, scan (normal to the layers) through the second harmonic of the modulation (at q, = 0.069 Å⁻¹). The peak width, limited by the sample mosaic, set a lower limit on the coherence of the modulation of at least 1000 Å. Although there is no evidence that the in-plane positional order of the molecules is correlated across the water layers, the long wavelength modulation is three dimensionally ordered.

In the Lβ' region, instead of finding one phase, as was expected, we found three distinct phases that we have labeled LβF, LβF, and LβF (the subscripts coming from thermotropic liquid crystal nomenclature [see (8) and references therein]. The average tilts of the chains in these phases, as determined from the scattering, is shown in Fig. 4 (top). In all three phases the in-plane positional order extends ~200 Å and is not correlated across water layers. The magnitude of the chain tilt increases continuously with increasing RH from ~26° in LβF to 30° in LβF. The phases are distinguished by the direction of the tilt with respect to the local distorted hexagonal lattice. In LβF, the tilt is between nearest neighbors; in LβF, it is

![Fig. 2. Photographs of thick, freely suspended hydrated DMPC films observed in reflected white light in the Lα, LβF, and Pβ phases. The three Lβ phases are optically indistinguishable.](image-url)
toward a nearest neighbor; and in \( L_{\text{AL}} \), it varies continuously between the two, with second-order transitions on both ends. These phases are distinct by symmetry. The details of the scattering and the relation between real and reciprocal space structures are discussed elsewhere (13). In addition to the fact that \( L_{\text{p}} \) must now be distinguished by a subscript, it is also clear that a high degree of orientation of the layers is necessary to identify these phases. Schematics of the scattering in reciprocal space are shown in Fig. 4 (bottom). As one can see, poor alignment of the layer normal would cause the patterns to be smeared out along the curved line, causing the distinctions between the phases to be lost; thus, they remained undetected until now.

Oriented films in the \( L_{\text{n}} \) phase allow us to directly measure the magnitude of undulations of the bilayer membranes, which gives us information relating to the bending modulus of a bilayer \( (k_c) \) and the bulk compressibility of the bilayers (8) in that medium. Peierls (14) and Landau (15) were the first to recognize that 3D structures that are periodic in only one direction, such as the lyotropic \( L_{\text{n}} \) phase, are marginally stable to thermal fluctuations that destroy the long-range order and change the \( \delta \)-function (0,0,1) peaks from the layers to less sharp algebraic singularities whose form is asymptotically (16)

\[
S(0,0, q_z) = (q_z - q_m)^m - 1
\]

where

\[
q_m = \frac{\pi m^2 k_B T}{2 \rho^2 (B_k / d)^{1/2}}
\]

(\( k_B \) is the Boltzmann constant) near the peak positions \( (q_m = 2 \pi m / d, \) where \( m \) is an integer). This power law behavior for the first harmonic \( (m = 1) \) of \( S(q) \) has been confirmed in a thermotropic smectic-A system (17). More recently, the power law behavior and the scaling of \( q_m \) with \( m^2 \) has been confirmed for the first two harmonics of \( S(q) \) in a series of quaternary and ternary systems in the lyotropic \( L_{\text{n}} \) phases in randomly oriented samples (3).

Although the mean-square height variation of a bilayer diverges with the size of the sample, it does so only logarithmically and is given by (15, 16)

\[
\langle u^2 \rangle = \frac{2 \pi^2 \eta}{L^2} \frac{\ln(L/a)}{a^2}
\]

where \( L \) is the characteristic length over which the average is taken, and \( a \) is the in-plane interparticle distance. Using the full (nonasymptotic forms) of \( S(q) \), we can also determine \( k_c \) and \( B \) independently (3).

Scattering data from the first two harmonics in freely suspended films of the ternary system SDS, pentanol, and water in a sealed cell with the film in equilibrium with the vapor phase of the mixture are contrasted with data from the DMPC-water system in Fig. 5. In the ternary system, pentanol reduces the bending modulus, increasing undulations (18) and giving \( q_1 = 0.23 \pm 0.03 \) as determined by the statistical errors of a least-squares fit. For the pure DMPC system, the layers are much stiffer, so \( q_1 = 0.36 \pm 0.003 \). From these results we can estimate \( q_2^2 / q_1^2 = 0.09 \) for SDS-pentanol-water and <0.001 for DMPC (averaged over 1 \( \mu m \)). With this technique, the effect of various cosurfactants on the elastic properties of membranes can be directly probed.

In summary, we have demonstrated the power of the freely suspended film technique for lyotropic multimembrane systems by directly measuring the modulation orientation in the \( L_{\text{p}} \) phase; by distinguishing the three \( L_{\text{p}} \) phases \( (L_{\text{FF}}, L_{\text{AL}}, \) and \( L_{\text{AL}}) \); and by measuring the line shapes of well-oriented \( L_{\text{n}} \) peaks, yielding information on the elastic properties of the membranes. The DMPC experiments were done under controlled and continuously variable humidity. It is also possible to control the thickness of these films from hundreds of molecular layers down to a single bilayer in order to determine the effects due to the surface layers or finite thickness (6). In general, the alignment obtained with this technique is useful, if not essential, in measuring structure and correlations in systems that are weakly ordered, such as lyotropic membranes.

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Neonatal Hepatitis Induced by \( \alpha_1 \)-Antitrypsin: A Transgenic Mouse Model

Mark J. Dycaico, Seth G. N. Grant, Katherine Felts, W. Stephen Nichols, Stephen A. Geller, Jeffrey H. Hager, Amy J. Pollard, Steven W. Kohler, Heidi F. Short, Frank R. Jirik, Douglas Hanahan, Joseph A. Sorge*

Transgenic mouse lineages were established that carry the normal (M) or mutant (Z) alleles of the human \( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-Pi) gene. All of the \( \alpha_1 \)-Pi transgenic mice expressed the human protein in the liver, cartilage, gut, kidneys, lymphoid macrophages, and thymus. The human M-allele protein was secreted normally into the serum, particularly in hepatocytes, and was found in serum in tenfold lower concentrations than the M-allele protein. Mice in one lineage carrying the mutant Z allele expressed the human protein in the liver, cartilage, gut, kidneys, lymphoid macrophages, and thymus. The human M-allele protein was secreted normally into the serum in hepatocytes, and was found in serum in tenfold lower concentrations than the M-allele protein. Mice in one lineage carrying the mutant Z allele expressed the human protein in the liver, cartilage, gut, kidneys, lymphoid macrophages, and thymus.

The pathophysiology has been attributed to intracytoplasmic inclusions of \( \alpha_1 \)-Pi within hepatocytes (6). It is suspected that neonates with hepatisis are more likely to develop cirrhosis as adults. It is not known why only 15% of ZZ homozygotes develop hepatisis, although it has been suggested that increased liver damage in these individuals is caused by expression of higher levels of protein in liver cells. It is also not known why boys develop \( \alpha_1 \)-Pi hepatisis more often than girls by a 2:1 ratio. In adults, liver disease associated with \( \alpha_1 \)-Pi is seen primarily in the form of cirrhosis. About 17% of all adults with nonalcoholic cirrhosis have the MZ heterozygous genotype (7). Again, there is a 2:1 ratio of male to female \( \alpha_1 \)-Pi patients with nonalcoholic cirrhosis.

To study the effects of human \( \alpha_1 \)-Pi alleles, transgenic mice were produced that contain the human \( \alpha_1 \)-Pi M and Z genes. To increase the probability of regulated, high-level expression, genomic \( \lambda \) clones that contained a substantial amount of 5' flanking region were isolated (Fig. 1). A Sna B1–Eco RI fragment (6.1 kb) of the \( \lambda \) clone containing the M allele was exchanged with the same length fragment from a clone containing the Z mutation located in exon G (Fig. 1). Thus two clones, each containing 21.4 kb of human \( \alpha_1 \)-Pi sequence, were produced that differed at a single nucleotide responsible for the Z mutation. The presence of the Z mutation was confirmed by nucleotide sequencing.

The 21.4-kb Sal I fragments were gel purified and injected into fertilized C57BL/6J mice correctly housed by intercrossing B6D2F1/J (C57BL/6J × DBA/2J) mice (8).

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Fig. 1. Map of the human \( \alpha_1 \)-Pi genomic clone used to construct transgenic mice. Exons A and B and the macrophage-specific promoter (\( \rho_1 \)) have recently been discovered (17). Exon C was originally referred to as exon 1. \( \rho_1 \) is the hepatocyte-specific promoter. The distances between restriction sites are in kilobases. Genomic libraries were constructed in \( \lambda \) EMBL-3 from normal human DNA and from cell line GM3578, which is homozygous for the \( \alpha_1 \)-Pi Z allele. E, Eco RI; Sa, Sal I; Sna I, Sna B1.
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