research on the $^4A_2$ and $^2A_1$ states. Our preliminary work on these states showed that the $^4A_2$ H_2CC cation (with a singly occupied $2b_2$ orbital) has a very high activation energy for rearrangement and that the $^2A_1$ barrier is nearly zero at the DZP CISD level of theory. This is in good agreement with the results in a preprint from Baker.[22] The $^2A_1$ state suffers badly from spin contamination in Baker's work (based on the unrestricted Hartree-Fock method), and extrapolation of his values leads to the $^2B_1$ state being 7 kcal/mol higher than $^2A_1$. This is compared to our $^2B_1$ state predicted to be below $^2A_1$ by 2 kcal/mol using DZP CISD, making the order of these states problematic. Our activation energy for the $^2B_1 \rightarrow {}^2\Pi_u$ rearrangement converged to a value 3.6 kcal/mol higher than that from Baker's highest level of theory.

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FEATURE ARTICLE

Stability of Lyotropic Phases with Curved Interfaces

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The diverse structural forms of lipid-water phases are representative of those found in many lyotropic and amphiphilic systems. These structures consist of interfaces which divide the material into hydrophobic and hydrophilic volumes. The net result of a very complex set of intermolecular interactions is that the interfaces behave as surfaces endowed with a spontaneous curvature and whose separations are subject to constraints of molecular lengths and component densities. Phase transitions which result in an abrupt change of the curvature of the interfaces may be understood phenomenologically as a competition between the elastic energy of bending the interfaces and energies resulting from the constraints of interfacial separation. The application of this approach is reviewed for transitions between lamellar, hexagonally packed cylindrical, close-packed spherical, and bicontinuous cubic structures of diacyl biomembrane lipids. It is shown that the structural dimensions and phase transitions of the lipid mesomorphs may be largely understood in terms of the phenomenological model. The existence of a spontaneous curvature associated with the lipid monolayers of bilayers implies that biomembranes exist in a state of compositionally controlled elastic stress which may provide a chemically nonspecific rationale for the types of lipids found in cell membranes. A discussion is given of the evidence that membrane proteins are functionally sensitive to the elastic stress. The article concludes with a summary of related outstanding problems.

Introduction

At high concentrations in water, polar biomembrane lipids form liquid crystalline structures with an astonishing degree of geometrical complexity. Consider, for example, one of the cubic phases of glycerol monooleate-water mixtures (Figure 1); this is but one of the many liquid crystalline phases seen in the phase diagram of this system. The structure consists of two mutually interpenetrating, but separate, mesh works of water channels separated by a multiply connected bilayer wall of monooleate molecules organized on a three-dimensionally periodic cubic lattice.[1] This structure spontaneously self-assembles upon mixing of the constituents. Further, although the monooleate headgroups are confined to the hydrophobic-water interface and the water to the water channels, the molecules are in every other way in a fluid form and randomly diffuse over many unit cells of the structure each second. Even more remarkably, this structured liquid has unit cell dimensions considerably larger than the size of any of the constituent molecules even though there are no long-range forces present. What are the physical principles which control the structure of this and many other amphiphilic systems? As discussed in this article, in the past 5 years a surprisingly simple picture of the forces which are involved has emerged and has potent implications for areas as diverse as catalysis and biomembranes.

The lyotropic mesomorphism exhibited by glycerol monooleate is shared by many of the polar lipids commonly found in biomembranes. The word lyotropic indicates that the structures and phases which are formed are a function of the ratios of the constituent molecules, such as the water-to-lipid ratio, as well as the temperature and pressure. Mesomorphism, from the Greek mesos, or middle, indicates that there is a sequence of thermodynamically distinct phases sandwiched between the low-temperature solid phase and the high-temperature isotropic fluid phase. Typically, these mesophases are liquid crystalline; i.e., they simultaneously exhibit aspects of crystalline periodicity and liquidlike molecular diffusibility. An example of the phase sequence observed in a biomembrane-water mixture as a function of temperature is shown in Figure 2. Depending on the system chosen, the same phase sequence may be observed as a function of salt concentration, pressure, ratios of the constituent lipids, or pH. More generally, homologous sequences of similar structures are seen in diverse systems which exhibit amphipathic character, such as diblock copolymers. In the case of the diblock copolymers, lyotropic

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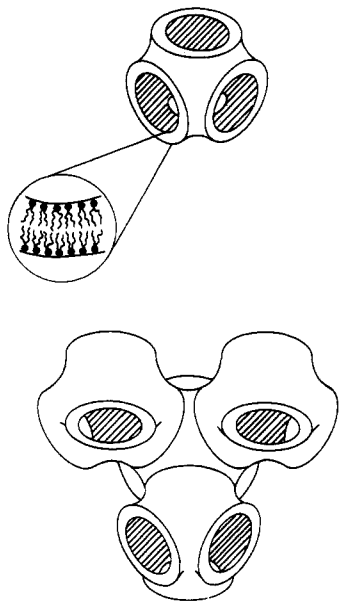

Figure 1. (a) Artist's representation of the repeating unit of a bicontinuous cubic phase with *Pn3m* symmetry. The centers of the four circular openings of the unit may be imagined as forming the vertices of an inscribed tetrahedron. The walls of the unit consist of a bilayer (inset) with water on either side of the wall. The units stack together as shown in (b). The midsurface of the bilayer wall falls on or near a minimal surface for which the mean curvature is zero everywhere. Note that in the interests of visual clarity the bilayer wall has been drawn as thin. In real lipid cubic phases the lipid concentration may exceed 50%. From ref 22.

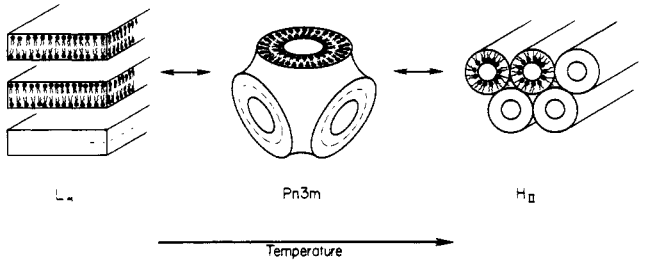

Figure 2. The mesomorphic sequence of phases which is observed as the temperature of a lipid–water system is raised is lamellar to bicontinuous cubic to inverse hexagonal. Molecules are shown schematically on some of the edges of the lipid layers. The volumes which are not filled with lipid molecules are filled with water. A similar mesomorphic sequence is observed with diblock copolymers, in which case one block substitutes for the lipid and the other block substitutes for the water. Bicontinuous cubic phases do not appear in all systems (see text), but when they do appear, it is always between lamellar and hexagonally packed cylindrical phases.

variation is accomplished by varying the lengths of the mutually insoluble polymeric chains which comprise the blocks, and the polymer molecules themselves play the role of both the amphiphiles and the solvents.

The fact that sequences of structurally homologous phases are seen in diverse systems in which the intermolecular interactions are quite different suggests that an understanding of the phase behavior should be sought at a phenomenological level which subsumes a wide variety of microscopic forces. An example of such a phenomenological understanding is the nature of amphiphilic behavior itself: At a molecular level, oil and water do not mix for very different reasons that liquid polystyrene and liquid polyisoprene do not mix. Ultimately, the tendency to mix can be specified by a phenomenological mixing parameter which involves the energy required to insert a molecule of one component (e.g., oil or polystyrene) into a bulk liquid of the other component (e.g., water or polyisoprene). Once the mixing parameter is known, much amphiphilic behavior may be understood simply by considering the competition between microsegregation, which lowers the free energy of the system, and the entropy of mixing, which tends to finely intermingle the molecular constituents. Indeed, most of the classical literature on surfactant aggregation is concerned with this competition. As a result, the existence of a critical micelle concentration is reasonably well understood. If the energy of mixing is very high, or if the constituents are present in comparable amounts, large-scale microsegregated aggregates, such as those shown in Figure 2, typically form. The more general question of the shapes of the aggregates which result is more subtle: Each of the aggregates of Figure 2 is microsegregated so that the respective immiscible components form microdomains which contact one another only along an interface. To understand why each of the aggregates of Figure 2 forms in sequence, we must come to an understanding of the factors controlling the shape of the interfaces.

A phenomenological understanding which is sufficiently broad to explain the homologous phase sequences observed with both biomembrane lipid–water and diblock copolymer systems is only beginning to emerge. To date, the primary interest of the group at Princeton has been the elucidation of the phase behavior of biomembrane lipid–water systems, especially for phase transitions which result in a change of the curvature of the lipid–water (e.g., hydrophobic–hydrophilic) interface. In consequence, a good qualitative understanding of the competing forces which drive lipid transitions has been obtained. This understanding is also shedding light on the problem of biomembrane lipid diversity, namely, the problem of identifying the factors which determine the lipid makeup of membranes in living systems.

Interfacial Curvature Altering Lipid-Phase Transitions

The immediate goal is to understand the phase transitions which result in alteration of the curvature of the lipid–water interface of polar biomembrane lipids. These molecules typically consist of a relatively small polar headgroup attached to one, or more commonly, two hydrophobic, hydrocarbon tails. Examples include soaps, phospholipids, and lipids in which the headgroups are carbohydrate units.

Polar lipids exhibit many kinds of phase transitions. In a series of classic papers, Luzatti and co-workers used X-ray diffraction to study the diverse mesomorphic forms in the water concentration–temperature phase diagrams of a variety of lipid systems.[2] Most of the literature on lipid-phase transitions is concerned with the melting of the lipid hydrocarbon tails, the so-called gel to "liquid crystalline" transitions, which represent only a small portion of the transitions on the lyotropic phase diagrams. (Note that in this context "liquid crystalline" refers to a phase in which the chains are in a melted, fluctuating state.) The physics governing chain-melting transitions is known to be dominated by chain–chain interactions, is reasonably well understood, and, therefore, will not be discussed explicitly in this article. Rather, our concern will be with transitions in this melted chain state which result in a change of the curvature of the lipid–water interface, such as those of Figure 2. The emphasis will be on systems in which the transitions occur primarily as a function of temperature.

Lipids are large molecules with many internal degrees of freedom and subject to many kinds of molecular interactions. In order to simplify the problem and to identify the relevant phenomenological parameters, the strategy which was taken was to ignore all interactions except those which involved a sudden change in energy at curvature-altering phase transitions. The lamellar to inverse hexagonal, or L_α to H_{II} (Figure 2), transition was selected for analysis because it is geometrically simple and was the subject of much current biological interest. The biological interest is a consequence of the realization, largely inspired by the work of Pieter Cullis and co-workers,[3] that very roughly half of the lipid species commonly found in biomembranes undergo this transition when purified and hydrated under physiological

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(3) Cullis, P. R.; Hope, M. J.; deKruijff, B.; Verkleij, A. J.; Tilcock, C.P.S. In *Phospholipids and Cellular Regulations*; Kuo, J. F., Ed.; CRC Press: Boca Raton, FL, 1985; Vol. 1.

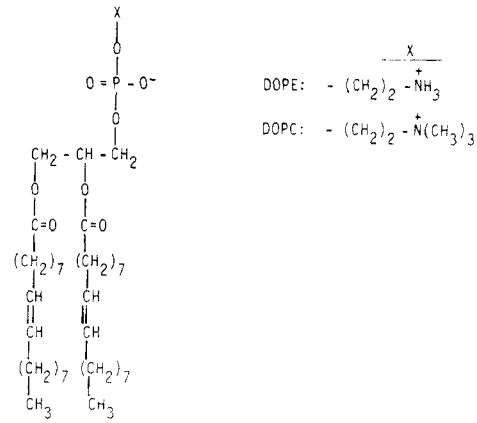

Figure 3. Chemical formulas for DOPE and DOPC.

conditions. We might call these lipid species H_{II}-prone because they readily form H_{II} phases. These are to be distinguished from lamellar- or bilayer-prone lipid species which do not readily undergo the L_α–H_{II} transition. Our concern, then, was to identify parameters which can be used to distinguish between lipid mixtures which readily undergo the transition and those which do not. If one understood factors which distinguish H_{II}-prone from bilayer-prone lipid mixtures, then one might understand why the lipid mixtures characteristic of biomembranes usually contain large fractions of H_{II}-prone lipids.

An example of the subtle differences which differentiate H_{II}-prone from lamellar-prone lipids is given by comparison of the water-rich portions of the phase diagrams of dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylethanolamine (DOPE; Figure 3). These lipids differ only in the degree of methylation of the quaternary nitrogen. At 2 °C both DOPE and DOPC are in a lamellar phase. (All H_{II}-prone lipids are in a lamellar phase at sufficiently low temperatures.) However, as the temperature is raised beyond about 6 °C, DOPE undergoes the transition to the H_{II} phase. DOPC remains in the L_α phase to well above 100 °C.

How is this difference to be understood? It had been known for many years that lipid systems which form mesophases with curved interfaces do so because this tends to minimize a bending energy of the lipid monolayer. This spontaneous tendency to bend may be characterized in a number of ways. The most intuitively appealing characterization is to visualize the shape of the volume occupied by a molecule in the H_{II} phase as "tapered", i.e., smaller in cross-sectional area at the headgroup than at the tail. This may be quantitatively specified by a dimensionless shape parameter given by v/al, where v is the molecular volume, a is the area at the lipid–water interface, and l is the length of a tail.[4] Such a "shape concept" characterization is often misunderstood and leaves much to be desired. Rigorously speaking, one must refer to the shape which minimizes the overall free energy of a given molecular volume under a given set of conditions. This bears only a weak resemblance to the steric shape of the molecule because lipids are highly flexible and because factors such as charge, hydrogen bonds, etc., strongly affect the free energy. If v/al is taken to be a characterization of the shape of the mean volume actually assumed in a given phase, then v/al is simply a tautological description of the phase and has no predictive value, because the shape changes sharply at the phase transition. Therefore, v, a, and l should not be taken to refer to the actual molecular dimensions, but rather to the dimensions which the system would prefer in the absence of other constraints; this poses serious problems of definition of v, a, and l and of measuring the values for real systems. Finally, it is difficult to see how to apply v/al to lipid mixtures.

The approach taken at Princeton was to simply accept that a complex of lipid monolayer depth dependent forces can yield a monolayer with a spontaneous curvature. The in-plane forces at the hydrophilic surface of a lipid monolayer include electrical charge, hydrogen bond, and other interactions which generally are different from those at the hydrophobic surface. The resultant forces are usually functions of the molecular area at the depth of the monolayer in question. If the sum of the in-plane forces is balanced for an area which is larger at the tail end than at the headgroup end, then the monolayer may have an effective moment which yields a minimum-energy configuration in which the monolayer is bent with a concave headgroup surface. In this case, the monolayer is said to have a spontaneous curvature.[5] Helfrich,[6] in characterizing the bending energy of asymmetric bilayers, showed that the free energy of bending a thin layer with a spontaneous curvature, C_0, is given by

$$E = (k_c/2)(C_1 + C_2 - C_0)^2 + k_g C_1 C_2 \qquad (1)$$

where E is an energy per unit area (effectively, an energy per lipid molecule), C_1 and C_2 are the principal curvatures, and k_c and k_g are constants called the rigidity and Gaussian curvature constants, respectively.[7] Note that the energy of $(k_c/2)(C_1 + C_2 - C_0)^2$ is minimal when $C_1 + C_2 - C_0 = 0$; i.e., the surface is elastically relaxed when the net curvature is equal to the spontaneous curvature. Also, the principal curvatures are physical curvatures of the surface while the spontaneous curvature is a thermodynamic property of the monolayer which has the dimensions of a curvature. Alternatively stated, the spontaneous curvature is that value which self-consistently minimizes the first term of the right-hand side of eq 1 for a given system.

The above description of a lipid monolayer makes numerous assumptions and is an oversimplification of what may occur in a real system. It treats the system as a continuous material which exhibits only a single dominant bending moment. Ideally, eq 1 can be generally applied for layers which are mathematically thin and which have curvatures which deviate from C_0 by only a small amount. However, a priori, there is no reason to believe that the description of the last paragraph does not describe the dominant energetics of a lipid monolayer. The approach which was taken was to assume that the monolayers *do* have a well-defined spontaneous curvature and to proceed to examine the consequences which lend themselves to experimental confirmation. As shall be seen, the spontaneous curvature description is, in fact, measurable and usefully describes many lipid monolayers.

An important advantage of a spontaneous curvature description is that the spontaneous curvature is a colligative property of the monolayer. Thus, the spontaneous curvature, C_0 (or, equivalently, its inverse, the spontaneous radius of curvature, $R_0 = 1/C_0$), can describe a variety of lipid mixtures.

The spontaneous curvature is simply a formal mechanism for describing the tendency of a monolayer to bend to a particular curvature and, as such, introduces no new real physics over earlier descriptions of curved phases. The most significant new contribution from the Princeton group was the realization that while a spontaneous curvature might explain why a monolayer curls up, for instance, in an H_{II} phase, it does not explain the phase transitions, e.g., why the monolayer suddenly flattens in undergoing the H_{II} to L_α transition. Consider, for example, the thermally induced L_α to H_{II} transition of DOPE in excess water. One approach toward understanding this transition is to assume that the spontaneous curvature suddenly changes from zero (flat bilayers) to large values (small H_{II} cylinders) at the transition temperature and that the shapes of the mesomorphs follow to

(4) Israelachvili, J. N.; Marcelja, S.; Horn, R. G. *Q. Rev. Biophys.* **1980**, *13*, 121.

(5) This approach has long been used in liquid crystal theory. See, for instance: de Gennes, P. G. *The Physics of Liquid Crystals*; Oxford University Press: London, 1974. Stephen, M. J.; Stralley, J. P. *Rev. Mod. Phys.* **1974**, *46*, 617.

(6) Helfrich, W. *Z. Naturforsch.* **1973**, *28C*, 693.

(7) The curvature of a line at a given point is defined as $1/R$, where R is the radius of the best fitting circle which is locally tangent at that point. Surfaces are describes by two curvatures, called principal curvatures, which represent the maximum and minimum (signed) inverse radii of the best fitting circles. The sign of the radius changes if the fitted circle moves from one one side to the other of the surface; in this way surfaces which are convex or concave have curvatures of different signs.

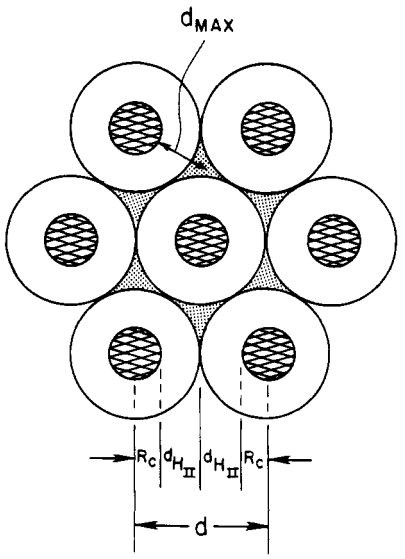

Figure 4. Structural dimensions used in the text are defined for cross sections through an H_{II} phase. The cross-hatched region represents water cylinders of radius R_c, while the circles and the stipled areas represent the lipid hydrocarbon chains. The chain packing energy arises from the fact that in H_{II} phases chains have to extend to different lengths to fill the hydrocarbon region. The mean variation in chain extensions is at least from d_{max} to $d_{H_{II}}$. In multilamellar phases (not shown), the d spacing is the distance from the center of a bilayer to the center of the neighboring bilayers.

minimize the bending energy. However, there are no obvious features in the statistical mechanics of lipids which might cause such sudden large changes in C_0. An alternative explanation, which was adopted, was that there are competing geometry-dependent contributions to the overall free energy of the monolayer such that the curvature energy and these competing terms cannot always be simultaneously minimized. The phase transition results when the sum of these terms is reduced by a change in the monolayer geometry. The advantage of such an approach is that it can involve purely smooth and continuous changes of underlying free energy contributions to the overall free energy. Kirk et al.[8] considered electrostatic, hydration, and hydrocarbon packing energies as competing terms. In the interest of clarity, let us focus on the competition simply between curvature and hydrocarbon packing (to be explained). These terms shall be seen to dominate in the case of electrically neutral lipids with large water spaces and in the presence of excess water. This, as it turns out, is the case for DOPE–DOPC mixtures.

Liquid crystalline lipid chains, by definition, contain gauche rotamers about some of the saturated carbon–carbon bonds, leading to an effective shortening of the molecule relative to the all-trans configuration. Each gauche rotamer costs about 0.5 kcal/mol but also increases the number of configurations available to the chain. Thus, the chains exhibit polymer-like properties whereby the number of gauche rotamers, and, hence, the mean molecular length, is determined primarily by a competition between the energy of introducing gauche rotamers and the resultant increase in entropy of the chains. Very much akin to the case with polymers, an isothermal extension or reduction of the mean length of the tail is expected to entail a elastic energy cost. In the lamellar geometry, and with a single chain species, there is no geometrical constraint which dictates that the mean chain length for any molecule need be different from any other; this, in fact, largely determines the thickness of the hydrophobic region of the bilayer. However, in the H_{II} phase, given uniformily curved (i.e., cylindrical) water cores, certain of the tails have to reach further than others simply to fill the hydrocarbon lattice (Figure 4) at near uniform density. Therefore, all the molecules cannot be at a free energy minimum with respect to chain extention. We call this the hydrocarbon packing price paid whenever the two opposed lipid water interfaces are not parallel to one another.

The inherent competition between lamellar and curved mesophases now becomes clear. If the monolayer is endowed with a spontaneous curvature of the sign just discussed, then the monolayers can lower the free energy with respect to bend by curling toward the spontaneous radius of curvature, R_0. In doing so, however, it incurs a rise in the free energy associated with packing the hydrocarbon chains. Thus, the lamellar phase has a low energy with respect to hydrocarbon packing and a high energy with respect to bending of the monolayer. The H_{II} phase has a high energy with respect to packing of the chains but a low energy with respect to bend. In general, these two free energy contributions depend differently on temperature. The L_α to H_{II} phase transition can occur if there is a temperature at which the sum of the two energies in a curved geometry falls below the sum in a lamellar geometry.

One more piece of the puzzle is required before the picture which has just been described can be subjected to experimental test, namely, an understanding of why the excess water H_{II} phase always occurs at a higher temperature than the lamellar phase. If one considers the stippled area of the H_{II} phase hydrocarbon shown in Figure 4 as representative of the strained chains and the thickness of the annulus surrounding the water cores as approximately the "relaxed" lipid chain length, then a trivial calculation shows that both the maximum extension of chains required to reach into the center of the stippled area and the fraction of the hydrocarbon in the stippled area are both growing as the first power of the radius of the central water core. In other words, for an approximately constant mean monolayer thickness, the hydrocarbon packing energy is growing with the radius of the central water core. Larger water cores cost more chain packing energy. As shall be seen, the spontaneous radius of curvature generally falls with increasing temperature. Thus, as the temperature increases, the free energy drop incurred by curving (e.g., consider eq 1) is growing even as the magnitude of the hydrocarbon packing price which would have to be paid in an H_{II} phase is falling. Both factors make the H_{II} phase energetically more favorable as the temperature rises.

It is worth noting that the hydrocarbon packing energy breaks the symmetry between H_I and H_{II} phases. In an H_I phase, commonly seen with single-chain surfactants, the headgroups coat the outside of the lipid cylinders and water is in between the cylinders. The predominant belief in the field has been that an understanding of the physics of the H_I phase directly translates to an understanding of the H_{II} phase in that the phases differ primarily by the sign of the spontaneous curvature. Indeed, the two phases do have curvatures of the opposite sign, but the H_{II} phase has no direct analogue of the hydrocarbon packing energy of the H_{II}. A direct consequence of this is that the H_I-phase lipid cylinders are rotationally symmetric about the cylinder axis whereas those in the H_{II} phase have 6-fold symmetry.

The model of the L_α–H_{II} phase transition which has just been described, i.e., that curvature competes with hydrocarbon packing, is plausible but, when critically examined, is seen to depend on a number of assumptions about the behavior of lipid monolayers. One, therefore, desires to perform experimental tests which check the assumptions and the predictions of the model. DOPC and DOPE were chosen as test systems for examination. The procedure was to use small-angle X-ray diffraction to probe for both the phase behavior and the dimensions of the observed phases as the temperature or composition was varied.

A few comments about the experimental methods are in order: First, the speciments were unoriented dispersions of either known water fractions or, more commonly, in excess water. In the latter case, since water permeates readily through lipid monolayers, the amount of water in the liquid crystal was not a constraint. As opposed to many charged surfactants, these lipids are zwitterionic and swell to a limited degree in excess water; the remaining water coexists as a bulk water phase. Second, in most of the data about to be presented, each data point was derived from a complete two-dimensional diffraction pattern. Literally thousands of dif-

(8) Kirk, G. L.; Gruner, S. M.; Stein, D. L. *Biochemistry* **1984**, *23*, 1093.

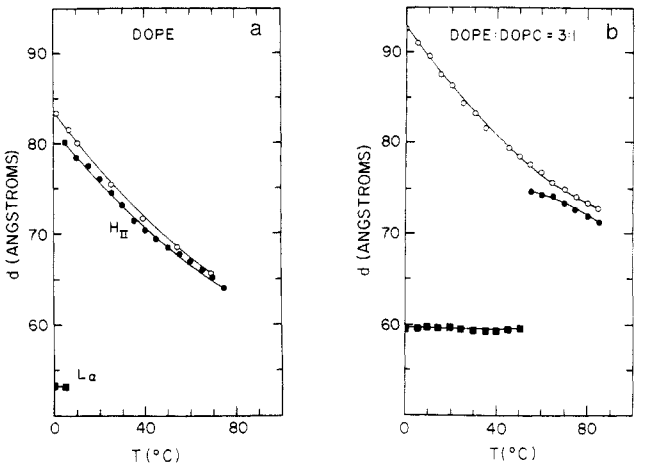

Figure 5. (a) The phase behavior and d spacings of DOPE in excess water are shown as a function of temperature. Squares represent L_α phases and circles are for H_{II} phases. In the absence of alkane (solid symbols) the phase transition occurs in the vicinity of 5 °C; in the presence of several percent alkane (open symbols), the transition is below 0 °C. (b) The phase behavior of DOPE:DOPC = 3:1 is similar, except the larger spontaneous radius of curvature of the mixed phospholipid system increases the d spacing and causes the transition temperature in the absence of alkane to be raised to the vicinity of 55 °C. Figure modified from ref 10.

fraction patterns were required. These studies would have not been feasible were it not for the efficient, highly automated apparatus developed in our laboratory.[9] Finally, since the model to be tested depends on competitions which are functions of the structural dimensions, the X-ray determined structural dimensions are of the utmost importance in understanding the systems. Other probes, such as optical textures or NMR, may have sufficed for the phase determinations but would not have yielded the dimensions. Also, X-ray diffraction is very conservative of the specimen material. If required, a full map of the behavior of a specimen over the temperature range of −30 to +90 to −30 °C in 5-deg increments could be done with less than a milligram of lipid and typically in less than a day.

Figure 5a shows the phases and unit cell basis vector lengths, d, of DOPE in excess water as a function of temperature. At roughly 5–10 °C, DOPE undergoes the L_α to H_{II} transition. Note that the d spacing of the H_{II} phase (d and the other structural parameters are defined in Figure 4) decreases monotonically as the temperature increases. Over the entire temperature range shown, the thickness of the lipid annulus decreases by only about 1 Å;[9] almost all of the decrease in d is due to a decrease in the radius of the central water core, R_c, which, as shall be seen, is about equal to the spontaneous radius of curvature, R_0, less a displacement. In terms of the model given above, the transition occurs because if H_{II} tubes occurred below the observed transition temperature, the d spacing would be on an extrapolated curve of the H_{II}-phase d spacing, corresponding to very large central water cores. But recall that the free energy of packing hydrocarbon grows with the size of the water core. At some point it is energetically favorable to simply pay the price in curvature energy and not in chain packing energy; this point determines the bilayer to hexagonal phase transition temperature, T_{bh}. Therefore, a perturbation of the system in a way which lessens the hydrocarbon packing energy would lessen the competition, in favor of the H_{II} phase, and the transition temperature would be expected to drop.

Fundamentally, the hydrocarbon stress is due to the fact that the lipid chains are anchored at the lipid–water interface and have to have different mean lengths to fully fill the hydrocarbon zone. Figure 5a also shows the effect of the addition of a few percent of a short alkane, such as dodecane. Short alkanes mimic chains but are not anchored to the interface. Therefore, one might expect that they would preferentially partition into regions where the lipid chains are stressed since this allows the lipid chains to relax, thereby relieving the hydrocarbon packing stress which is competing against expression of the spontaneous curvature, and cause T_{bh} to drop. This is exactly what happens. Note that the d spacing in the presence of dodecane does not show any discontinuities at the transition temperature in the absence of alkane and that the d spacings are close to those seen without alkane. The former observation suggests that the spontaneous curvature extrapolates smoothly below the T_{bh} observed in the absence of alkane, while the latter observation suggests that dodecane has little effect on the spontaneous curvature. A priori, we have no reason to believe that a given hydrophobic substance should affect chain packing without also strongly affecting R_0. Fortunately, it appears that, for the lipids in question, C10 to C14 alkanes primarily affect the packing energies. This is not generally true for other purely hydrophobic substances. Finally, note that "preferential partitioning" of the alkane into the stressed zone does not imply that the alkane is confined to that location. Rather, one conjectures that it spends a bit more time in the stressed location relative to other areas where it would otherwise be found. However, this has not yet been directly proven by diffraction because experiments aimed at localizing a small concentration increase of the alkane in any particular part of the hydrophobic zone are very difficult to perform and have not yet been successful.

In contrast to DOPE, DOPC in excess water is in the L_α phase over the entire −15 to 85 °C temperature range. In terms of the model, DOPC has a very large spontaneous radius of curvature, which would correspond to very large central water cores. This presents an overwhelming hydrocarbon packing price. However, since the spontaneous curvature is a colligative property of the monolayer, mixtures of DOPE and DOPC might be expected to be characterized by a R_0 value between the extremes of the pure constituents. Figure 5b shows the d behavior of a DOPE:DOPC = 3:1 mixture. Note that as opposed to pure DOPE, T_{bh} is about 55 °C and that above this temperature the H_{II} d spacing is considerably larger than that of DOPE. Other mixtures show that at a given temperature in the H_{II} phase the d spacing grows with the DOPC fraction and that the changes are almost entirely due to changes in the water core radius.[10,11] Thus, a continuum of R_0 values can be obtained from the appropriate mixtures, a feature which is very useful in experiments on the spontaneous curvature. As with pure DOPE, the addition of a few percent dodecane relieves much of the hydrocarbon packing constraint and allows expression of the spontaneous curvature, i.e., an H_{II} phase, down to 0 °C. Such an enormous change in transition temperature for such a small addition to the total hydrophobic mass is hard to explain by alternative models which rely on molecular volume or shape alone.

On the basis of the model, one would predict that T_{bh} should fall if the lipid mixture contains a small amount of lipid with extralong chains. This was shown to be true by mixing small amounts of long-chain phosphatidylcholines (PCs) into a DOPE–DOPC mixture.[12] The addition of PC is also expected to increase the spontaneous curvature, thereby raising T_{bh}. However, T_{bh} is very sensitive to the amount of added hydrocarbon beyond the mean chain length and only moderately sensitive to increases in R_0. Thus, it is not surprising that the addition of long-chain PCs to the lipid mixture simultaneously increases the

(9) Reynolds, G. T.; Milch, J. R.; Gruner, S. M. *Rev. Sci. Instrum.* **1978**, *49*, 1241. Gruner, S. M.; Milch, J. R.; Reynolds, G. T. *Nucl. Instrum. Methods* **1982**, *195*, 287. Templer, R. H.; Gruner, S. M.; Eikenberry, E. F. *Adv. Electron. Electron Phys.* **1988**, *74*, 275. Kirk, G. Thermodynamic Models and Experimental Investigations of the Lamellar (L_α) to Inverse Hexagonal (H_{II}) Phase Transition of Lipid–Water Systems. Ph.D. Thesis, Princeton University, 1984. Tate, M. W. Equilibrium and Kinetic States of the L_α–H_{II} Transition. Ph.D. Thesis, Princeton University, 1987. Gruner, S. M. Presented at the Synchrotron Radiation Conference, Tskuba, Japan, 1988; *Rev. Sci. Instrum.* **1989**, *60*, 1545.

(10) Kirk, G. L.; Gruner, S. M. *J. Phys. (Les Ulis, Fr.)* **1985**, *46*, 761.

(11) DOPC-rich DOPE–DOPC mixtures are not stable in the absence of sufficient alkane. Given limited alkane, these mixtures separate into coexisting L_α and H_{II} phases in which the DOPE to DOPC ratio adjusts to the amount of alkane (Rand, R. P.; Fuller, N.; Gruner, S. M.; Parsegian, V. A., *Biochemistry*, in press).

(12) Tate, M. W.; Gruner, S. M. *Biochemistry* **1987**, *26*, 231.

d spacing and decreases T_{bh}. Again, this is very difficult to explain unless curvature and packing are largely distinct contributions to the overall free energy.

A detailed examination of the structures of the H_{II} phases of DOPE and DOPE–DOPC mixtures reveals that, indeed, the large changes in H_{II} d spacing seen with temperature and with the ratio of DOPE to DOPC are largely due to changes in the size of the central water core radius.[13] Changes in the thickness of the lipid annulus ($d_{H_{II}}$ in Figure 4) are limited to about an angstrom. Likewise, the addition of dodecane or tetradecane has very small effects on $d_{H_{II}}$.[10,14]

Qualitatively, the measurements described so far imply the existence of a spontaneous curvature but do not indicate the amount of energy required to bend the monolayers. Is, for example, eq 1 a realistic indication of the bending energy? For planes or cylinders, one of the principal curvatures is always zero, so eq 1 reduces to

$$E = (k_c/2)(C - C_0)^2 \quad (2)$$

where C is the nontrivial curvature. As long as there is a unique minimum in the bending energy, over a sufficiently restricted range of curvatures about the minimum at C_0, one of course expects such a variation. However, the energy must deviate from the parabolic form at some values of $(C - C_0)$, and it is useful to ask where this occurs. An experiment designed to measure the bending energy as a function of the H_{II} cylinder radius is described in the next paragraph. The basic rationale of the experiment is the observation that the H_{II}-phase water core is often 40 Å or larger in diameter. Since chemical hydration of the headgroups is unlikely to contribute significantly to the overall energy beyond a few water diameters from the headgroups, one expects that much of the water can be removed simply by paying the elastic energy price required to curve the monolayer to a smaller diameter. In other words, much of the water in the H_{II} phase is simply there to fill the volume of the cylinder centers.

Imagine a chamber which is divided in two by a semipermeable membrane which is transparent to water but not to lipid or to a hydrophilic polymer, such as poly(ethylene glycol) (PEG). Completely fill one chamber with lipid in the H_{II} phase. Fill the other chamber with PEG solution. The polymer competes for water with the H_{II} phase and, because of the water transparency of the semipermeable membrane, both chambers can be brought into equilibrium with each other. One can measure the osmotic pressure of the PEG solution and in situ measure the dimensions of the H_{II} water core by X-ray diffraction. If the PEG concentration is raised, i.e., the osmotic pressure is raised, the radius of the H_{II} water core shrinks due to the competition for the water. Now, the water core radius, for a given amount of lipid, is uniquely related to the volume of water per lipid molecule. By repeating the experiment at many different osmotic pressures, one can obtain a curve of osmotic pressure vs volume of water per lipid molecule in the H_{II} phase. But the integral under a pressure vs volume diagram over a range of volumes is just the work required to change the volume over that range. This can be graphed as work vs water core radius. One now does a least-squares fit of this data to eq 2 and determines the range of radii over which the fit is good. Over such a range, the values of k_c and $C_0 = 1/R_0$ are then determined by the fit.

This experiment was performed[15] for three systems, DOPE, DOPE with tetradecane, and DOPE:DOPC = 3:1 with tetradecane, and the results were recently refined and extended.[11] In practice, the semipermeable membrane was not needed because the polymer will not enter the H_{II} phase and there was also a more elegant way to do the analysis—but these are minor details. The results are very interesting. First, there exists a surface about a third of the way down the lipid chains from the headgroup where the cross-sectional molecular area changes little when the monolayer is osmotically bent. There is an inherent ambiguity in eq 2 in situations where the thickness of the layer is comparable to the radius of curvature: at what depth in the layer should the radius be defined to? By choosing the surface which has the least area change upon bending, one uniquely specifies R. Further, since this surface was nearly constant in area, eq 2, which is an energy per unit area, is unambiguously also a specification of the bending energy per molecule. This definition of R also generated the best fit to eq 2 over the widest range of osmotic pressures. Second, the values of k_c thus obtained (1.2 to 1.7 × 10^{-12} erg; see ref 11), when scaled to bilayers, agree well with bilayer values of k_c obtained by other methods, thus lending confidence to the procedure.

One of the original goals, namely, finding a parameter which measures the "H_{II}-prone" tendency of a lipid system, has to some degree been achieved: R_0 measures this tendency among comparable systems. For example, for DOPE, DOPC, the nitrogen-methylated analogues of DOPE, and mixtures of these lipids, in the presence of adequate dodecane to express the H_{II} phase in excess water, R_0 is a good measure of the H_{II} tendency in the absence of dodecane. Larger R_0 values correspond to higher H_{II} transition temperatures. R_0 should not be expected to be an absolute measure whereby any two diverse lipid systems can be compared because the energetic competition of the transition involves, at the very least, other parameters such as k_c and constants describing the chain stretch energy. At present, there is little knowledge of how these latter parameters vary among diverse lipid systems. Also, the situation is complicated by molecules, such as the long-chain lipids which affect both curvature and packing. Even given these caveats, much progress has been achieved in the general understanding the L_α–H_{II} transition.

An understanding of the functional form of the chain stretch energy is in particular need of attention. How is the energy to be scaled from one kind of lipid chain to the next? It is likely that a detailed molecular model may be required to answer this question. Some indications of factors which might be important were obtained in a recent study of the phase behavior and structural dimensions of PEs with a wide variety of chains.[16] The study included chains of various lengths with both cis and trans double bonds, with various degrees of branching at the chain ends, and with cyclopropane rings in the end of the chains. Although the gel to liquid crystalline transition, T_m, varied widely, the span of T_{bh}–T_m was almost constant for chains of the same effective chain length (i.e., the number of carbon atoms along the main chain). Further, for chains of a given effective chain length, the d spacings of the single-phase L_α and H_{II} phases on either side of the L_α–H_{II} transition were the same. This suggests, somewhat surprisingly, that the chain packing energy is primarily a function of the effective chain length, not the chain volume. Whether this is true or not is actively being explored.

Other Lipid Phases

The understanding of the L_α–H_{II} transition gained so far extends to other transitions which involve changes in the shape of the lipid–water interface. For example, it was formerly thought that cubic phases consisting of close-packed inverted (water-cored) spherical micelles occurred in systems consisting of a single lipid and excess water. The spontaneous curvature vs chain packing description suggests that this phase does not occur because the excluded volume between close-packed spheres is a larger fraction of the hydrocarbon volume than between close-packed cylinders. Therefore, for a given curvature, the H_{II} phase would always be of lower overall free energy.[8] Recent diffraction and NMR data indicate that the cubic phases are, in fact, geometrically complicated bicontinuous cubic structures, such as shown in Figure 1.[17]

(13) Tate, M. W. Equilibrium and Kinetic States of the L_α–H_{II} Transition. Ph.D. Thesis, Princeton University, Princeton, NJ, 1987. Tate, M. W.; Gruner, S. M. *Biochemistry*, submitted for publication.

(14) Gruner, S. M.; Tate, M. W.; Kirk, G. L.; So, P. T. C.; Turner, D. C.; Keane, D. T.; Tilcock, C. P. S.; Cullis, P. R. *Biochemistry* **1988**, *27*, 2853.

(15) Gruner, S. M.; Parsegian, V. A.; Rand, R. P. *Faraday Discuss.* **1986**, *81*, 29.

(16) Lewis, R. N. A. H.; Mannock, D. A.; McElhaney, R. N.; Turner, D. C.; Gruner, S. M. *Biochemistry* **1989**, *28*, 541.

The same competition which dominates the transition behavior of the L_α–H_{II} phase transition can be used to understand geometrically complicated cubic phases such as the one shown in Figure 1. This phase is one of several known cubic mesomorphs which have the crystallographic symmetry and the topology[1,18] of periodic minimal surfaces (PMS). PMS are three-dimensionally periodic surfaces which are free from self-intersections and which have zero curvature everywhere; i.e., every point on the surface is a saddle point for which the sum of the principal curvatures ($C_1 + C_2$) is zero.[19] This surface may be visualized by considering the surface traced out by the tips of the lipid chains of Figure 1, that is to say, the bilayer center. These surfaces divide space into two identical, intertwined but unconnected volumes. They are sometimes called bicontinuous cubics, because the hydrophilic and hydrophobic volumes are continuous throughout; in fact they are tricontinuous (one surfactant volume and two distinct water volumes). When these phases are observed with inverted phase lipids, e.g., lipids which will form H_{II} phases, they invariably appear in between the L_α and H_{II} phases in the phase diagrams.

It has been realized for several years that many lipid and surfactant cubic phases bear some relationship to PMS.[20] This was puzzling to me because the L_α–H_{II} phase transition is, by the model given above, a transformation of a zero-curvature phase to a curved phase which is driven by the expression of a spontaneous curvature. Given this, it seemed odd that other zero-curvature phases should intervene.

The key to understanding these phases was the recognition that the surface which is important for a curvature energy calculation is determined by the effective depth-dependent moment of the monolayer. As discussed above, the energy to bend a thick monolayer in the H_{II} phase is well-described by eq 2 if the radii are referred to the near-neutral or pivotal surface which tends to be a bit down the chains from the headgroups. Thus, a calculation of the curvature energy of the lipid monolayers of Figure 1 should not be carried out over the minimal surface but over the pivotal surfaces which are removed on either side of the minimal surface by most of the thickness of the monolayer chain region.

Surfaces which are quasi-parallel to a minimal surface but a fixed distance to either side of it are not minimal but, rather, are nearly coincident with the surfaces of constant nonzero mean curvature which have been recently discussed by Anderson.[21] Surfaces of constant mean curvature are ones for which $C_1 + C_2$ are everywhere equal to a constant value; the minimal surface is the special case where the constant is zero. Anderson et al.[22] showed that the distance variation between the two constant mean curvature surfaces on either side of a minimal surface can be very small. In particular, for a given monolayer specifying a given pivotal surface there exists a value of curvature for which the average distance between the corresponding constant mean curvature surfaces is equal to the distance between the pivotal surfaces of the opposed monolayers. If two constant mean curvature surfaces, of curvature C, are nearly coincident with the pivotal surfaces of two monolayers which have a spontaneous curvature $C = C_0$, then the resulting mesomorphs are a better compromise between curvature and hydrocarbon packing energies than either L_α or H_{II} phases. However, for a given monolayer if the spontaneous curvature is too small, then lamellar phases are better compromises. Likewise, for large curvatures, H_{II} phases win out.[14] As is the case for L_α and H_{II} phases, the cubics are governed by the competition between curvature and packing. No new physics is necessarily required to explain these peculiar cubic phases!

Given the previous paragraph, one might ask why PMS cubics are not observed between all L_α and H_{II} phases. In many lipid systems, cubic phases form only after extended incubation or specimen manipulation. This suggests that PMS cubic phases might be kinetically difficult to form. The topologies of PMS cubic, L_α, and H_{II} structures are very different. Insofar as these systems are characterized by a different local topological genus, then it is impossible to transform between them without tearing the lipid–water interface. Such tears expose hydrocarbon chains to water and are thus expected to be energetically expensive. Although the transition shapes of the interfaces which occur when the liquid crystal undergoes phase transitions are not known,[23] it is reasonable to expect that the kinetic reticence of PMS cubic phase formation involves the number of interfacial tears required. In other words, PMS cubic phases might, indeed, exist as free energy minima between L_α and H_{II} phases but may be kinetically hindered.

Shyamsunder et al.[24] examined this possibility by thermally cycling DOPE across the L_α–H_{II} transition rapidly many times. A cubic phase had never been reported in this part of the DOPE phase diagram. After several hundred cycles sharp lines appeared in the X-ray diffraction pattern which did not index as either lamellar or hexagonal lattices. Upon continued cycling, the lines grew in intensity at the expense of the L_α and H_{II} diffraction lines until the entire pattern was consistent with a cubic lattice of the form shown in Figure 1. Other L_α to H_{II} transforming lipids exhibited similar behavior. Once the cubic lattices were formed they were very difficult to destroy. However, the systems could be reset by repeated deep cooling and mechanical mixing. It was suggested that lattice defects introduced during the L_α–H_{II} phase transition accumulated to a density sufficient to form a kinetically hindered cubic phase which, once formed, was kinetically very stable. The generality of these results has yet to be explored, but we speculate that similar behavior will be seen in most L_α–H_{II} lipid systems.

The cubic phases have two nonzero principal curvatures, so eq 1, no eq 2, generally applies. In particular, a realistic description of the curvature energetics involves the rightmost term of eq 1, the Gaussian curvature term. Thus far, this term has been neglected and it has been shown that the remaining term is sufficient to explain the qualitative sequence of L_α–cubic–H_{II} phases which are observed. This is not meant to imply that the Gaussian term is unimportant; indeed, inclusion of it may be necessary to explain the quantitative behavior, such as the unit cell size of cubic phases. Unfortunately, the value of the Gaussian constant, k_g, is not even roughtly known. Understanding of the consequence of Gaussian curvature is a very important area for future work.

Understanding is also lacking with respect to the sequence of cubic phases which are observed in a number of lipid systems. Several cubic phases with the topology of periodic minimal surfaces are known,[18-20] and phase transitions occur between them as a function of water concentration and temperature. The different PMS cubic forms have different surface area to volume values, and this is certainly an important factor in determining the phase observed as a function of the water concentration.[25] However, transformations between different PMS-type cubic phases are also observed as a function of temperature in excess water conditions under which the water volume to lipid surface area is not a constraint. Much more work will be needed before cubic phases are well-understood.

The enthalpies which are seen in transitions between cubic phases are typically very small. Further, disordered assemblies are often associated with cubic transitions and well-ordered cubic phases are difficult to obtain. This may indicate that there exists a series of cubic phases of nearly the same free energy, surrounded

(17) However, the above argument does not preclude close-packed inverted spherical micelles in lipid–water–oil systems, nor does it take Gaussian curvature contributions into account.

(18) The X-ray phase problem has been solved for only a few cubic mesomorphs. (For example, ref 1. See also reference in: Luzzati, V.; Mariani, P.; Gulik-Krzywicki, T. In *Physics of Amphiphilic Layers*; Meunier, J., Langlvin, D., Boccara, N., Eds.; Springer: Berlin, 1987.) Typically, the structure is inferred from chemical constraints and the crystallographic symmetry.

(19) Hyde, S. T.; Andersson, S. *Z. Kristallogr.* **1984**, *168*, 221.

(20) Charvolin, J. *J. Phys. (Les Ulis, Fr.)* **1985**, *46*, C3-173.

(21) Anderson, D. M. Studies in the Structure of Microemulsion. Ph.D. Thesis, University of Minnesota, 1986.

(22) Anderson, D.; Gruner, S. M.; Leibler, S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5364.

(23) See, however: Siegel, D. P. *Chem. Phys. Lipids* **1987**, *42*, 279.

(24) Shyamsunder, E.; Gruner, S. M.; Tate, M. W.; Turner, D. C.; So, P. T. C. *Biochemistry* **1988**, *27*, 2332.

(25) Hyde, S. T.; Andersson, S.; Ericsson, B.; Larsson, K. *Z. Kristallogr.* **1984**, *168*, 213.

by high activation barriers which are probably related to the need to disrupt the integrity of the hydrophobic–hydrophilic interfaces.[24]

In closing this section, I note that the emphasis thus far has been on thermotropic transitions which occur in lyotropic systems but in the presence of coexisting excess bulk water. The situation is somewhat different in true lyotropic transitions where the amount of water constrains the lattice structure. As mentioned above, with limited water an additional factor which is important with cubic phases, such as that shown in Figure 1, is the interfacial area to component volume ratio. The constant mean curvature surfaces have the property that they minimize the interfacial area with respect to surfaces which can be generated by local perturbations and which preserve the volume of the three continuous compartments.[21] A comprehensive theory which accounts for both the lyotropic and thermotropic behavior of lipid systems has not yet been formulated. But the success so far in understanding thermotropism suggests that the major ingredients of such a theory may have been identified.

Biological Implications

The spontaneous curvature picture of lipid-phase behavior, as described above, has important implications for biomembranes. There has been interest in understanding the biological role for H_{II}-prone lipids since the realization that such lipids comprise a large fraction of the bilayers of typical biomembranes. Possible roles for such lipids tend to fall into four categories. First, there is the possibility that H_{II} phases actually occur in living systems and perform specific functions. The evidence for H_{II} phases in healthy cells is weak; consequently, this is not likely to be a major reason as to why these lipids are so prevalent. A second possibility which has been suggested is that the presence of a large H_{II}-prone lipid fraction endows the bilayer with a transient mesomorphic capability which assists in instances where the continuity of the lipid–water interface must be disrupted. Events which require such a disruption, such as membrane fusion, endocytosis, and cell fission, are quite common in the life of cells. This may, indeed, be an important role for H_{II}-prone lipids. Unpublished studies from our laboratory indicate that whole lipid extracts from many biomembranes are characterized by R_0 values which are just to the lamellar side of bilayer stability. However, it will be difficult to prove that H_{II}-prone lipids have an important transient mesomorphic role until the basic processes in question, such as endocytosis, are better understood. A third possible role for H_{II}-prone lipids, advocated by Pieter Cullis, is that an increase in the fraction of H_{II}-pronte lipid in the biomembrane affects the permeability of the membrane to ions and small molecules. Relatively little work has been done on this possibility.

A fourth possibility is that important proteins are sensitive to the presence of H_{II}-prone lipids. Navarro et al.[26] showed that the efficiency with which the Ca^{2+} pump of sarcomplasmic reticulum membranes couples ATP hydrolysis to Ca^{2+} pumping, when the protein is reconstituted in vesicles, increased with the fraction of the lipid which is H_{II}-prone. Moreover, the effect appeared to be chemically nonspecific in that it mattered little as to which H_{II}-prone lipid was used. Similar nonspecific H_{II}-prone lipid enhancement of activity has been reported for other membrane protein systems.[27]

An important consequence of the phase transition work summarized in this paper is that it is now feasible to assign a quantitive measure to the degree to which a lipid mixture is "H_{II}-prone". In 1985 I suggested that R_0 may be a homeostatically regulated property of biomembranes and that if this is so then there must exist proteins which are sensitive to R_0, if only to maintain the regulation.[28] The suggestion was motivated by the observation that lipid extracts from biomembranes are often perilously close to the L_α–H_{II} transition and that the composition must be regulated so as to not overstep the edge and render the bilayers too mesomorphically unstable. It was suggested that homeostatic regulation of R_0 might be investigated by measurement of the R_0 values of bacterial membranes for those bacteria which can be made to be fatty acid auxotrophic and whose lipid fatty acid composition can, therefore, be altered. Figure 6 shows the result of such an experiment.[29] Note that the upper temperature out of the L_α phase is remarkably constant over a wide range of fatty acid and lipid headgroup compositions, suggesting a constant R_0 value. Note also that the temperature at which the transitions occur is well above the 37 °C growth temperature of the organisms, so the actual occurrence of the phase transition is not directly involved.

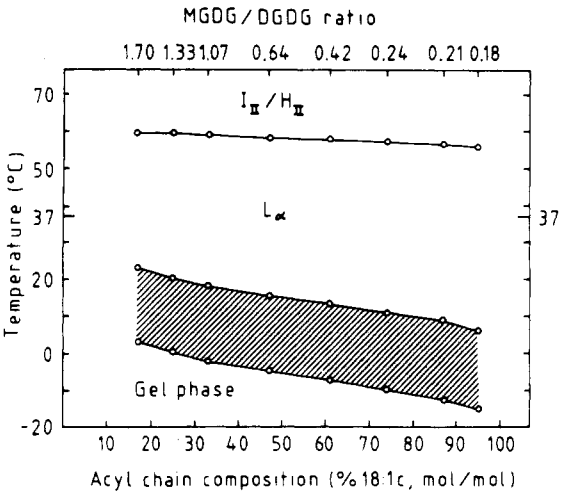

Figure 6. Phase equilibria of lipid mixtures extracted from membranes of *A. laidlawii* bacteria which have been grown at 37 °C in the presence of various ratios of palmitic and oleic fatty acids. Under suitable conditions, the bacteria respond by incorporating the fatty acids into their lipid chains in a way which varies with the fatty acid ratio of the culture media. The two major lipids produced are monoglucosyldiglyceride (MGDG) and diglucosyldiglyceride (DGDG). The lower x axis shows the mole percent of palmitoyl and oleoyl chains in the polar lipids. The upper x axis shows the metabolically obtained MGDG:DGDG ratios. The total lipid content is not represented. The hatched area denotes the gel to liquid crystalline phase transition interval as determined by ESR. Above the border of the L_α phase, no lamellar phases were found. Two- and three-phase regions (L_α, H_{II}, and I_{II} = cubic) occur just below this border (within less than 10 °C). Note that the phase transition temperature out of the L_α phase is roughly constant and well above the growth temperature of the bacteria. From ref 29.

It was also suggested[28] that the sensitivity of important membrane proteins to R_0 may also provide another, although less direct, test of the R_0 regulation of biomembranes. Specifically, if cells regulate R_0 so as to optimize the function of important membrane proteins, then when such proteins are reconstituted into lipids (within the lipid plasticity range of the native membranes), protein function should correlate with R_0 of the reconstituted lipids. It will be of great interest to examine whether the function of selected integral membrane proteins correlates with the R_0 value of the imbedding bilayer. If this proves to be so, then an understanding of the L_α–H_{II} transition will have led to a measurable property which characterizes the H_{II}-prone tendency of certain lipid mixtures and allows a quantitative analysis of the earlier cited studies which report on a correlation of protein function and the fraction of H_{II}-prone lipid.

One can only speculate as to the mechanism whereby proteins might couple to R_0 because the structure, much less the mechanism, is not known for any of the proteins in question. However, a general mechanism whereby a conformational engine buried within the monolayers may exchange reversible work with the lipid environment has been known to us since our early studies on the H_{II} phase:[8] A bilayer which is comprised of monolayers which

(26) Navarro, J.; Toivio-Kinnucan, M.; Racker, E. *Biochemistry* **1984**, *23*, 130.
(27) Jensen, J. W.; Schutzbach, J. S. *Biochemistry* **1988**, *27*, 6315. Wiedman, T. S.; Pates, R. D.; Beach, J. M.; Salmon, A.; Brown, M. F. *Biochemistry* **1988**, *27*, 6469.
(28) Gruner, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3665.
(29) Lindblom, G.; Brentel, I.; Sjoland, M.; Wikander, G.; Wieslander, A. *Biochemistry* **1986**, *25*, 7502.

have small values of the spontaneous radius of curvature is in a highly elastically strained state. This ultimately arises because the area per lipid molecule in the headgroup region is too large relative to the area per molecule in the hydrocarbon region, given the molecular interactions which are present. Now imagine a protein which is buried in the monolayer and which is able to perform conformational changes which either vary the protein cross-sectional area in the polar region relative to the area in the hydrophobic core or vary the interactions with adjacent lipid molecules which leads to the spontaneous curvature, or both. For instance, the protein might consist of a cylinder of bilayer-spanning α-helices arranged like the staves of a barrel. Conformations which expand the protein area at the depth of the headgroups relative to the area in bilayer core reduce the available area per lipid headgroup and, thus, are able to extract work from the monolayers. Reversal of this conformational change is then an energy-consuming step. In terms of the protein barrel, a suitable conformational change is a breathing mode of the barrel. Obviously, other suitable conformations can be envisioned.

An order of magnitude estimate of the upper limit of energy available to the protein may be derived from eq 1: The energy per lipid molecule[30] required to completely flatten a monolayer cylinder originally bent to its spontaneous radius of curvature is $K_c/2R_0^2$. For $K_c \simeq 1.5 \times 10^{-12}$ erg and $R_0 = 30$ Å, this corresponds to an energy change of about 6×10^{-14} erg/lipid, which is about the value of k_BT at room temperature. This is the change in curvature energy if the intermolecular interactions were changed from those characteristic of the nonbilayer lipid to those which characterize the strongly bilayer lipid. Let us assume that a conformational change in the protein causes such a modification of the intermolecular interactions with the lipid immediately adjacent to the protein. The number of lipid molecules affected will, of course, vary with the protein, but an interaction with several lipid molecules may result in an energy change which considerably exceeds k_BT.

Ultimately, the most important message pertaining to the biological implications of monolayer spontaneous curvature is that a small R_0 bilayer is in a very energetically different state than a large R_0 bilayer even though the two may have the same thickness, surface charge, and "fluidity". Thus, liquid crystalline bilayers may differ in a way that has not previously been considered in experiments which address the structure and function of biomembranes. Moreover, this difference is chemically nonspecific in that it may be arrived at by many different mixtures of constituent lipids. The effects of this difference on biological experiments have not been carefully considered. The evidence cited above suggests that the effects may be significant in certain cases. One cannot help but note that the saturated PCs which are the workhorses of biophysics lead to large R_0 bilayers which appear to be far removed from the barely bilayer stable R_0 values typical of many biomembrane lipid mixtures.

Finally, the effects of proteins on the membrane R_0 values are not known. An understanding of the effects of protein is important because roughly half of the mass of a typical biomembrane is protein. Studies which have measured the effect of protein on the L_α–H_{II} transition by determining the change in T_{bh} upon the insertion of protein have generally failed to distinguish between equilibrium and kinetic effects. For instance, since the L_α–H_{II} transition occurs at the interface between adjacent bilayers,[31] it can presumably be kinetically inhibited by proteins which simply act as molecular spacers by keeping bilayers apart. In this case, the real equilibrium state, and the minimum curvature stress of the lipid monolayer, is not readily determined. The mechanism which we have used, namely, adding alkane to express the curvature in an H_{II} phase from which R_0 may be measured, is not useful in the presence of most proteins because the proteins are too large to enter the H_{II} lattice. A probe of elastic strain which works in bilayers is needed. Other factors which have yet to be critically analyzed in applying curvature concepts to real biomembranes are the asymmetry of biomembrane bilayers and the coupling of monolayers by membrane spanning proteins.

(30) This should be understood to only be an estimate because eq 1 is unlikely to be true over the full range of curvatures from $C = 0$ to $C = 1/R_0$.
(31) Gruner, S. M.; Rothschild, K. J.; de Grip, W. J.; Clark, N. A. *J. Phys. (Les Ulis, Fr.)* **1985**, *46*, 193.

Conclusion

Study of the competition between monolayer elastic curvature and hydrocarbon chain packing factors has yielded much insight about the mesomorphic behavior of lipid–water systems. Much remains to be done within the framework of the elastic curvature approach. I conclude by listing some remaining problems of importance:

1. The physical significance of the Gaussian (rightmost) term of eq 1 is unclear. It is not known whether this term modulates the phase behavior. The value of k_g is not known.
2. Factors which determine phase transitions between different PMS cubics need to be understood.
3. A true lyotropic theory must involve other known contributions to the overall free energy, such as electrical charge and hydration energies. Although these effects were considered by Kirk et al.,[8] the approach was relatively unsophisticated and is in need of improvement. In particular, it is unlikely that R_0 is independent of large values of electrical or hydration stress.
4. The phenomenological approach described in this paper has limits, of course, but it is not known where the limits become important. The spontaneous curvature energy is basically a continuum materials concept which must yield to a molecular picture at some as yet undetermined length scale. In particular, a molecular approach toward the determination of R_0 is needed. A likely byproduct of such an approach is insight into the coupling of R_0 and the hydrocarbon packing energy and a better, more quantitative understanding of the origin of the chain stress energy.
5. The phase sequences in systems as diverse as block copolymers and lipid dispersions are similar, as pointed out in the introduction, which suggests that an understanding of one system will help in understanding the others. However, this has yet to be demonstrated.
6. The picture, as presented so far, is an equilibrium approach. The actual transitions are often limited by kinetic factors, such as the energy of disrupting the lipid–water interface and the geometry of the transition pathways. More work is needed on kinetics.
7. The shapes of lipid mesomorphic structures are usually inferred from lattice symmetries. More direct and detailed structural information is needed.
8. A comprehensive phenomenological theory which simultaneously explains the thermotropic and lysotropic behavior of lipid systems is needed.
9. The biological importance of curvature stressed bilayers must be examined. Does it affect protein function? Do living membranes control curvature stress? If so, then such control must be considered as a factor in understanding biomembrane lipid diversity.
10. The synthetic systems examined to date all have symmetric bilayers. Biological membranes have an asymmetric lipid composition. How does lipid asymmetry affect mesomorphic transitions? Synthetic asymmetric bilayer systems are needed. In a similar vein, biomembrane bilayers are studded with bilayer spanning molecules, such as proteins. What are the effects of such molecules?

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