Membrane Models and Model Membranes

3. Membrane Models and Model Membranes

I. MEMBRANE STRUCTURE

Lipid Properties in Membranes

Lipids form a variety of extended, noncovalent structures that include the fundamental lipid bilayer of the membranes of cells. Many of the examples in this chapter will be illustrated with phospholipids, a class of polar lipids. Phospholipids are amphipathic molecules, with a hydrophobic portion (the hydrocarbon chains) and a hydrophilic portion (the polar head group). These properties are exploited to establish a hydrophobic barrier to permeability in close proximity to an aqueous medium. The permeability barrier is maintained by the association of lipids in structures that sequester the hydrocarbon portions in hydrophobic regions away from the aqueous medium. Only the polar headgroups then encounter the polar phase. By so doing, the phospholipid molecule can satisfy the hydrophobic effect, which is the dominant "driving force" behind membrane assembly.

Interesting properties result from such organizations of amphipathic molecules. An appreciation of those properties is key to understanding the behavior of lipids in cell membranes. How the amphipathic lipid molecule elects to achieve these properties can be partially rationalized, although not uniquely, by an interplay between thermodynamics and the chemical properties imparted by the structure of the lipids. Several different interesting lipid assemblies can be formed. Each morphology is stabilized by a balance among favorable and unfavorable interactions. The nature of the interactions is determined by the lipid structures. Therefore, one can expect differences in lipid covalent structures to lead to differences in the morphologies of the assemblies of lipids that result.


A. Phase Structures

1. Lamellar (Bilayer) Phase

The lamellar phase (lipid bilayers) is formed by the common lipids of biological membranes. In this structure, the polar heads of the lipids face the aqueous phase on both sides of the bilayer, and the hydrocarbon chains oppose each other inside the bilayer, as seen in Fig. 3.1. As an example, most common species of phosphatidylcholine form this phase.

One representation of the phospholipid bilayer comes from X-ray diffraction measurements. Phospholipids can be induced to form bilayers that stack, producing a repeating unit that can give rise to diffraction.\(^1\)

X-ray diffraction data have been used to establish the electron density profile across a phospholipid bilayer. A representation of such an electron density profile appears in Fig. 4.1.\(^2\) It is symmetrical because, in this case, the phospholipid bilayer is symmetrical in composition. When proteins are incorporated into the membrane, this symmetry is destroyed. The middle of the membrane has the lowest electron density. The membrane midplane contains the terminal methyl groups of the phospholipid hydrocarbon chains, which are experiencing considerable motional freedom. The points of highest electron density are near the outside edges of the membrane. This is the comparatively electron-dense region of the phosphate of the phospholipid. Thus it is possible to obtain the thickness of the membrane from the electron density profile by measuring the phosphate-to-phosphate distance. This is not far from the true membrane thickness because the polar headgroups of the phospholipids lie predominantly parallel to the membrane surface. The width so derived, from phosphate to phosphate across the membrane, is about 46 Å in the gel state for dipalmitoylphosphatidylcholine.

One can see a change in this width if the length of the hydrocarbon chains of the lipids is altered. In other words, the width of the bilayer changes in direct proportion to changes in the effective length of the hydrocarbon chains. For example, the width of the bilayer decreases going from a gel state to a liquid crystal state, or from a gel state 18:0,18:0 PC to a gel state 14:0,14:0 PC. These observations lend further support to the concept of a bilayer of lipid in membranes.

A simple means of understanding the packing of lipids into a lipid bilayer can be obtained by considering the shape of the molecule. For phospholipids like phosphatidylcholine, the cross-sectional area of the headgroup is similar to the cross-sectional area of the hydrocarbon chains. Thus the shape of the molecule can be approximated as a cylinder. If one considers that these cylinders are to be packed to protect the hydrocarbon regions from contact with water, the packing of a lipid bilayer becomes inevitable (see Fig. 4.2). This can be represented more quantitatively by

\[
P_r = \frac{A_h}{A_c}
\]

where \(A_h\) is the effective cross-sectional area of the headgroup, and \(A_c\) is the effective cross-sectional area of the hydrocarbon chain region, and \(P_r\) is the packing ratio. A lipid bilayer should result when \(P_r = 1\). As will be seen later in this chapter, modulation of the relationship between the cross-sectional area of the headgroups and the cross-sectional area of the hydrocarbon chains will lead to a modulation in the packing of the lipids.

Also interesting is the effect of unsaturation. The thickness of the membrane

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.1.png}
\caption{Representation of an electron density profile derived from X-ray diffraction for dipalmitoylphosphatidylcholine. The location of the electron-dense phosphates can be seen clearly as the prominent positive peaks. Reprinted with permission from Y. K. Levine, \textit{Prog. Surf. Sci.} 3, (1973): 279.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.2.png}
\caption{Schematic representation of how an approximately cylindrical shape of an amphipathic lipid \((P_r = 1)\) packs spontaneously into a bilayer configuration.}
\end{figure}
brane is decreased upon an increase in unsaturation, chain length otherwise being equal. The decrease in thickness reflects a reduction in the average chain length if a cis double bond is introduced. Furthermore, an increase in unsaturation from no carbon–carbon double bonds to one double bond increases the cross-sectional area of the molecule. Thus with an increase in unsaturation, a phospholipid will increase the surface area it occupies and decrease its average length, thereby preserving its total molecular volume.

The bilayer is one of the basic elements of biological membrane architecture. As mentioned previously, most phospholipids in membranes inhabit a bilayer structure, and many of the properties of pure phospholipid bilayers mimic the properties of cell membranes. Consequently, this particular lipid structure will be the center of attention for much of the remainder of this chapter.

However, in some cases, variations in the structure of the lipid headgroup and its consequent hydration produce sufficient stress on the fabric of the bilayer structure that some lipids tend to adopt alternate morphologies. Prior to examining the lamellar phase in more detail, therefore, this survey of the phase structures accessible to amphipathic lipids will be completed.

2. INTERDIGITATED BILAYERS

A variation on the normal bilayer structure has recently been described. Under certain conditions, the lipid hydrocarbon chain termini are not all located in the bilayer midplane. Hydrocarbon chains from one leaflet of the bilayer may overlap hydrocarbon chains from the opposing leaflet of the same bilayer. This is called interdigitation. Some of the possible interdigitated structures are presented in Fig. 4.3.3

In Figs. 4.3a and 4.3b, two different, fully interdigitated forms are schematically represented.4 One structure is made possible by a large difference in the effective length of the two hydrocarbon chains of the lipid. The effect is to locate one of the chain termini near the interface of the hydrophobic and hydrophilic regions of the bilayer. In the other fully interdigitated structure, both chain termini are near that interface. The thickness of the membrane in this latter structure approximates the length of one lipid molecule, rather than two as in the normal bilayer.

In both of these fully interdigitated bilayers, the lipid headgroups are required to guard more (than in a normal bilayer) of the bilayer surface against contact between the aqueous phase and the hydrophobic interior of the membrane. Such structures would thus appear to be favored by large, well-hydrated lipid headgroups. In this case, \( P_1 > 1 \) (or the headgroup can be reasonably altered in conformation to make \( P_1 > 1 \)).

The partially interdigitated bilayer schematically presented in Fig. 4.3c does not depart as strongly from the normal bilayer as do the fully interdigitated forms. In this case the polar headgroups are not required to cover significantly greater surface area than in a normal bilayer. Again, this form is favored by large differences in the effective length of the hydrocarbon chains. As in the other interdigitated forms, this partially interdigitated bilayer also would be expected to lead to a membrane that is thinner than the normal phospholipid bilayer.

To date, extended interdigitated bilayers have been found mostly in the gel state. Some of the strongest evidence for their formation has come from X-ray diffraction measurements of bilayer thickness. One example of a phospholipid that appears to form a partially interdigitated form in the liquid crystalline state is 18:0,10:0 PC, based on the large difference in effective chain lengths. Some of the sphingolipids, which in their natural form can exhibit large differences in effective chain length of the two hydrocarbon chains, would also be good candidates for interdigitation.

Transient interdigitation may be more widespread in the liquid crystalline state than is now appreciated. Two-dimensional nuclear Overhauser effect \(^1\)H NMR experiments of liquid crystalline bilayers from several
groups have provided evidence of dipolar interactions between phospholipid headgroups and terminal methyls of phospholipid hydrocarbon chains. One way to explain such data is through transient interdigitation.

It is somewhat premature, given the state of the field, to speculate heavily on what roles bilayer interdigitation might play in membrane biology. However, it is worthwhile to keep in mind that membrane thickness has been demonstrated to modulate membrane enzyme activity (see Chapter 7). And the potent platelet-activating factor (PAF) is a candidate for interdigitation.

3. MICELLAR PHASE

In the case of lipids that contain well-hydrated headgroups and only a single hydrocarbon chain, packing problems in the hydrocarbon region (or bilayer interior) lead to sufficient stress on the lipid bilayer that an alternate structure forms. With only one hydrocarbon chain, these lipids would have difficulty filling all the volume of the interior of a bilayer, while accommodating the area per headgroup forced on the molecule by the hydration of the lipid headgroup. In this case, \( P_t \gg 1 \). This molecular shape can be approximated by a cone.

The geometric constraints on the packing of cones lead to the formation of a sphere. The resultant sphere would have a diameter roughly equal to the length of two lipids. The interior of this sphere is a hydrophobic domain. This spherical structure is called a micelle. The structure of a micelle is illustrated schematically in Fig. 4.4. Lysophosphatidylcholine and many common detergents are well-known examples of lipids that form micelles.

Lipid micelles represent a molecular assembly in which the individual components are thermodynamically in equilibrium with monomers of the same species in the surrounding medium. Even though part of the molecule is hydrophobic, these lipids do have a finite solubility in the aqueous phase. The extent of the solubility is, of course, determined by unfavorable entropy contribution due to the ordering of the water structure by the hydrophobic moiety. The greater the hydrophobic surface area exhibited by that moiety, the fewer molecules that can be dissolved in the aqueous phase, and the lower the solubility of that lipid. The solubility of the lipid is also influenced by the polar nature of the headgroup, which should help accommodate the lipid in the water structure through binding of water molecules. The balance between these interactions and the unfavorable entropy contributions from the hydrocarbon chain of the lysolipid in water, for example, determines the solubility. It is interesting that from these considerations, one can predict that even a phospholipid should have a finite, if vanishingly small, solubility in water [for dipalmitoylphosphatidylcholine, the critical micelle concentration (CMC) = \( 10^{-10}M \)]. This phenomenon is important in some kinds of lipid movement between membranes.

Now consider the behavior of a micelle-forming lipid, like lysophosphatidylcholine, as a function of lipid concentration. At very low concentrations of the lipid, only monomers are present in true solution. As the concentration of the lipid is increased, a point is reached at which the unfavorable entropy considerations, deriving from the hydrophobic end of the molecule, become dominant. At this point, the lipid hydrocarbon chains of a portion of the lipids must be sequestered away from the water. Therefore, the lipid starts aggregating into micelles. The concentration at which this occurs is referred to as the critical micelle concentration (CMC). Table 4.1 provides CMC data for a number of detergents. The larger the size of the hydrophobic moiety, the larger the micelle and the lower the CMC.

Above the CMC, addition of further lipid to the system results largely in an increase in micelle population. At this point, the system is more properly referred to as a lipid suspension with coexisting populations of monomeric detergent and detergent micelles. Unlimited increases in micelle population and/or size are not possible. Therefore, a maximum in the concentration of lipid in the suspension can be reached. As an example a phase diagram for SDS appears in Fig. 4.5.
TABLE 4.1
Micellar Properties of Detergents

<table>
<thead>
<tr>
<th>Detergent</th>
<th>CMC (mM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>8.1</td>
<td>120</td>
</tr>
<tr>
<td>SDS (30 mM NaCl)</td>
<td>3.1</td>
<td>72</td>
</tr>
<tr>
<td>SDS (50 mM NaCl)</td>
<td>2.3</td>
<td>84</td>
</tr>
<tr>
<td>SDS (100 mM NaCl)</td>
<td>1.3</td>
<td>85</td>
</tr>
<tr>
<td>DTAB</td>
<td>15</td>
<td>61</td>
</tr>
<tr>
<td>DTAB (50 mM NaCl)</td>
<td>5.7</td>
<td>72</td>
</tr>
<tr>
<td>DTAB (100 mM NaCl)</td>
<td>4.4</td>
<td>73</td>
</tr>
<tr>
<td>DOC</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DOC (10 mM NaCl)</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>DOC (150 mM NaCl)</td>
<td>0.9</td>
<td>22</td>
</tr>
<tr>
<td>Cholate</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Cholate (10 mM NaCl)</td>
<td>5.1</td>
<td>3</td>
</tr>
<tr>
<td>Cholate (150 mM NaCl)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Taurocholate</td>
<td>2.8</td>
<td>4</td>
</tr>
<tr>
<td>Palmitolysoclethine</td>
<td>0.01</td>
<td>—</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>0.3</td>
<td>140</td>
</tr>
<tr>
<td>Octylglucoside</td>
<td>0.25</td>
<td>—</td>
</tr>
<tr>
<td>Laurylglucoside</td>
<td>0.2</td>
<td>—</td>
</tr>
<tr>
<td>C12E8</td>
<td>0.09</td>
<td>120</td>
</tr>
</tbody>
</table>

Micelles serve useful purposes in biology. Perhaps one of the most important is in the human digestive system. Bile salts are detergent species formulated by the liver from cholesterol and secreted into the intestine. These amphipathic molecules (an example of taurocholate is given in Fig. 4.6.) form micelles in aqueous media. In the bile, these bile salts are distributed among micelles, phospholipid vesicles, and monomers in solution. The bile is concentrated and delivered to the gut.7

The bile is secreted into the gut to aid in digestion. Cholesterol and triglycerides ingested as part of the diet have very low solubilities in water. This is due to the hydrophobic effect. However, these neutral lipids are soluble in hydrocarbon solvents. Bile salt micelles provide, in their interior, hydrocarbon-like droplets that are effective at solubilizing triglycerides (as well as the products of lipase hydrolysis) and cholesterol. Cholesterol mimics the bile salt by placing its hydroxyl near the polar-nonpolar interface, and burying the fused ring and hydrocarbon tail in the hydrophobic part of the micelle. Oriented in these micelles in the gut, dietary lipids are in a form acceptable as substrate to a number of digestive enzymes (lipases, etc).

Since detergents form micelles, they are useful in dissolving oily residues such as those found in the kitchen sink. Some other classes of detergents have important uses in membrane studies. As is discussed later in Chapter 6, many proteins are tightly bound to membranes and are insoluble in water due to the hydrophobic effect. Detergents are frequently used to solubilize these membrane proteins (see Chapter 7, Section V). The detergent solubilization process separates the proteins from the host membrane and can be used to purify the protein for membrane studies. The detergent achieves its solubilizing effect by binding to the hydrophobic surface of the protein and creating a detergent micelle around that hydrophobic portion. This protects against the interaction of the hydrophobic protein surfaces with the aqueous phase. The detergent thus takes the place of the membrane lipid and creates a soluble detergent–protein complex, suitable for a variety of biochemical studies.

The CMC of detergents is of obvious importance to their ability to solubilize membrane constituents since the detergents must be in a detergent micelle to be effective at solubilization. Additionally the CMC of the detergent may also play a role in the removal of the detergent in the course of a reconstitution experiment. Detergents can sometimes be removed by...
dialysis, which relies on the ability of detergents to exist as monomers in solution at a finite concentration. Detergent monomers penetrate the dialysis membrane, whereas membrane vesicles and detergent micelles cannot.

The magnitude of the CMC controls the effectiveness of the dialysis technique. If the CMC is high, then a high concentration of monomers can exist in solution. This can lead to a large chemical potential difference for the detergent monomer on the two sides of the dialysis membrane if the dialysate is low in detergent concentration. The influence of a large difference in chemical potential can lead to a rapid removal of detergent by dialysis, if the kinetics of removal are a simple first-order process involving transfer of the detergent across the dialysis membrane. Conversely, if the CMC is low, the removal of detergent by this technique is not effective.

However, a high CMC is not necessarily a good criterion for choosing a detergent for solubilization or reconstitution. If the CMC is too high, then the concentration of micelles at accessible total detergent concentrations is low. The effectiveness of partitioning of the hydrophobic molecules from the membrane into the detergent micelles is reduced by decreasing the effective volume into which such partitioning can take place. However, a low CMC may be useful for studying detergent binding to proteins. Therefore, the choice of a detergent is not an easy task.

Other methods for detergent removal are available. Gel permeation column chromatography can be used, analogous to a desalting experiment. For some detergents like Triton X-100, the use of a product like Bio-Beads (Bio-Rad Richmond, CA) can be helpful by providing a surface into which the detergent can selectively partition.

4. Hexagonal I Phase (H₁)

It was noted above that the micellar population in a detergent/water mixture cannot increase without limit as the detergent-to-water ratio increases. In fact, in the presence of low amounts of water, lipids that would normally form a micellar phase can form a larger aggregate. Long tubes of H₁ phase assemble, which can be thought of as many micelles fused together. These tubes have the polar headgroups facing out, and the hydrocarbon chains facing the interior. This phase structure is only seen under specialized conditions and is probably not particularly relevant to biological membranes.

5. Hexagonal II Phase (H₂)

In the above consideration of the assembly of lipids into structures satisfying the requirements of the hydrophobic effect, the amphipathic chemical structure of the lipids and the shape of the lipid molecules, the region of $P_r = 1$, $P_r > 1$, and $P_r > 1$, were explored. Yet to be explored is the region of $P_r < 1$. In this region, one would expect that inverse cone shapes would lead to an inverse packing, such as seen in the hexagonal II (H₁) phase. However, as will be seen below, the formation of the nonlamellar H₂ phase can be better understood in terms of the thermodynamics of hydration than on the basis of molecular shape.

Phosphatidylethanolamine is one of several common lipids derived from biological membranes that is capable of forming hexagonal II structures. The hexagonal II phase has attracted interest because of the significant portion of lipids of cell membranes that favor the H₁ phase when isolated from the other membrane components. The question that has to be asked is why biological membranes would incorporate into their structure lipids that destabilize the bilayer, which itself is essential to the structure and proper function of the cell membrane. This fascinating question will be addressed in part here and further in Chapters 7 and 9.

Lipid molecules in the H₂ phase pack inversely to the packing observed in the hexagonal I phase described above. The H₂ phase puts the polar headgroups on the inside and the hydrocarbon tails on the outside. An extended array of molecules packed in this way will form tubes. Since the polar groups must contact water, they surround on aqueous channel at the center of the tube (Fig. 4.7).

This structure therefore leaves an exposed hydrophobic surface on the outside of the tubes. Because of this hydrophobic surface, the tubes tend to pack closely together to exclude water from their outside surfaces. They stack like pipes being readied for a pipeline, forming an hexagonal array in cross section. This may leave a finite hydrophobic surface in probable contact with water on the outside of the collection of tubes. However, the otherwise energetically favorable packing apparently stabilizes this phase as a whole. Or an outer monolayer of lipid coats the surface of the collection of tubes to protect that hydrophobic surface from interaction with the aqueous phase.

The hypothesis of a spontaneous radius of curvature for lipid assemblies was introduced to help understand the tendency for lipids like PE to form the H₂ phase. This hypothesis states, in brief, that surfaces made up of lipids in a liquid crystalline state have a spontaneous tendency to form a surface of defined radius of curvature, $R_0$, called the spontaneous radius of curvature. Within this hypothesis, phospholipids like PC have a large $R_0$ and spontaneously form surfaces with relatively large radii of curvature, as is observed. However, phospholipids like PE have a relatively small, negative $R_0$ and thus have a tendency to form curved surfaces, such as in the H₂ phase. The coexistence of lipids with different $R_0$ values within
the same bilayer would be expected to lead to packing stress within the bilayer.

How is $R_0$ determined? The formation of the $H_{II}$ phase by PE and other lipids offers a means to measure $R_0$. Normally, the formation of the tubes of this phase leads to packing defects at the juncture of the tubes. Packing of cylinders in an hexagonal array leaves voids where each set of three tubes is in contact. Liquid crystalline hydrocarbon chains of the lipids can attempt to fill such voids, but this introduces stress into the packing.

Fig. 4.7. (A) Representation of the structure of the hexagonal II phase. (B) Freeze-fracture electron micrograph of the hexagonal II phase of dilauryl PE. Bar: 100 nm. Micrograph courtesy of Dr. S. W. Hui.

Considering the thermodynamics of the interaction of water with a membrane surface defined by PE headgroups leads to an appreciation of entropy terms contributing to $H_{II}$ phase formation. Phosphatidylethanolamino will be used as an example.

When in a bilayer, the headgroup of PE engages in intermolecular hydrogen bonds, $N-H$ to phosphate. This intermolecular hydrogen bond “neutralizes” the charges (positively charged amino and negatively charged phosphate) on the PE headgroup and competes effectively with water for binding to these charged groups. Fewer water molecules are bound by PE than by PC. Therefore, more unbound water molecules would be needed to cover the surface of a PE bilayer than a PC bilayer. Such water molecules must be ordered by the surface, giving rise to an unfavorable entropy term. Since many of these water molecules are not bound, little compensating enthalpy is available to contribute to the overall $\Delta G$. Therefore the interaction of the aqueous media with the PE surface is less favorable than the interaction of the aqueous media with the PC surface. This can be described in terms of the free energy of transfer, $\Delta G_p$, of the phospholipid headgroups into water as (the contributions of the hydrocarbon chains to this $\Delta G$ are ignored because they are expected to be virtually the same for the two phospholipids).
The discussion above suggests $\Delta G_{\text{HII}} < \Delta G_{\text{Lam}}$ and $\Delta S_{\text{HII}} > \Delta S_{\text{Lam}}$. Therefore, $\Delta G_{\text{HII}} > \Delta G_{\text{Lam}}$. In a sense, the surface of a PE bilayer is “hydrophobic” compared with the surface of a PC bilayer.

The structure of the phase formed by PE must therefore compensate for this hydrophobic effect. A structure that reduces the contact of the surface with water such as aggregation of bilayers will satisfy the need. Aggregation of PE bilayers, which excludes much of the water from between the bilayer surfaces, is commonly seen in aqueous dispersions of pure PE bilayers. An alternative means to compensate for the hydrophobic effect is formation of the HII phase structure. This structure packs the headgroups of the PE molecules more closely together, on the inside surface at the hexagonal tubes. Tight packing of the headgroups reduces their contact with the aqueous phase, thereby reducing the amount of ordered, but unbound water at the surface. Furthermore, the small diameter of the tubes of the hexagonal phase (~20 Å) reduces the total amount of water encountering the PE surface. From this one can deduce that the spontaneous radius of curvature, $R_0$, would necessarily be smaller for PE than for PC as is observed.

Hexagonal II phase is favored by PE dispersions at elevated temperatures. The effect of increased temperature is to increase the surface area occupied by each PE headgroup, thereby magnifying the unfavorable contribution to $\Delta G$ of the structure of the surface of PE bilayers. For similar reasons, unsaturation in the hydrocarbon chains (which also increases the surface area the headgroup must cover) favors the hexagonal II phase.

There is direct evidence for this role of the hydrophobic effect in the structures formed by PE.15 Chaotropic agents such as guanidine hydrochloride disrupt water structure, reducing the unfavorable nature of the interaction of a hydrophobic species with an aqueous phase. For example, guanidine hydrochloride can cause the unfolding or denaturation of proteins by stabilizing the interaction of hydrophobic amino acid side chains with the aqueous phase. With these considerations and the discussion above one would expect that chaotropic agents would stabilize the lamellar phase of PE. Such stabilization is observed experimentally (see Fig. 4.8). As mentioned above, addition of alkyl groups to the PE headgroup that do not interfere with the hydrogen-bonding capability of the amino group (such as the addition of an ethyl to C2 of the ethanolamine) enhances the hydrophobic character of the headgroup. Such modified PE more readily forms hexagonal II phase than the corresponding unmodified PE.

Fig. 4.8. 31P NMR spectra of aqueous dispersions of soybean PE. The spectra in the lower right represent pure lamellar phase. The spectrum at the top center represents pure hexagonal II phase. In the absence of the chaotropic agent the lamellar phase is unstable even at 15°C. Guanidine hydrochloride (GuHCl) stabilizes the lamellar phase in proportion to concentration added, as can be seen reading across the figure. In 3 M GuHCl, the lamellar phase is stable up to 55°C. This is the same concentration range of GuHCl that is required to denature soluble proteins, due to the ability of the chaotropic agent to alter the structure and properties of the aqueous phase.

What lipids form HII phase? Phosphatidylethanolamine with unsaturated hydrocarbon chains forms the hexagonal II phase readily. Diposphatidylglycerol in the presence of calcium is also capable of forming the hexagonal II phase,16 as is the glycolipid, monogalactosyldiglyceride, a component of the Acholeplasma laidlawii membrane.17 In the presence of calcium, the lipids of the retinal rod outer segment disk membrane form hexagonal II phase.18

In the region where an approximate balance is achieved between hexagonal II and lamellar phase, a third type of structure is sometimes formed. In freeze-fracture electron micrographs spherical particles, called lipidic particles, appear in the membrane bilayer19, 20 (Fig. 4.9). Although the structure of lipidic particles is the subject of controversy, the 31P NMR spectra show isotropic motional averaging for some of the phospholipids when lipidic particles are present. This has led some investigators to
postulate that lipidic particles are interlamellar attachments. Lipidic particles may be a nucleation point for formation of extended hexagonal II phase. Lipidic particles constitute discontinuities in the membrane that increase membrane permeability.

Figure 4.10 shows a phase diagram depicting the interplay of temperature and composition on the lamellar to hexagonal II phase transition of one mixed lipid system. An increase in temperature at a given composition favors formation of the hexagonal II phase. An increase in the phosphatidylethanolamine content at a fixed temperature also drives the system toward the hexagonal II phase. The phase diagram was constructed using three physical techniques. One is 31P NMR, where different powder patterns are observed for lamellar, hexagonal, and isotropic phases. The second is freeze-fracture electron microscopy, which is also sensitive to all three phases. The third is X-ray diffraction, which is sensitive to lamellar and hexagonal phase structures and provides definitive evidence of their existence. This study is an example of the use of several complementary techniques to obtain an adequate description of the lipid system.

Mixing bilayer-forming lipids with lipids capable of forming a hexagonal II phase can produce some provocative effects. For example, mixing a modest amount of a bilayer-forming phospholipid, like phosphatidylcholine, into phosphatidylethanolamine can change the thermodynamically favored state of the phospholipid mixture from the hexagonal II to the bilayer structure. The phase structure of the system then depends on the PC/PE mole ratio.

Other lipid mixtures can stabilize the bilayer. An example of bilayer stabilizing phase behavior can be seen in the mixing of lysophosphatidylcholine with fatty acids. If these two lipids are mixed in equimolar amounts, this mixture forms a bilayer. This structure results even though the individual components by themselves form micelles.
6. Cubic Phase

Another phase that can be exhibited by phospholipids is the cubic phase.\textsuperscript{25-27} This is nearly an isotropic phase, because phospholipids experience all possible orientations with respect to a laboratory reference frame on a relatively short time scale. However, the cubic phase is an extended structure and consists of short tubes connected in a hexagonal array. Cubic phase is sometimes found in mixtures of phospholipids that are changing from a lamellar phase to a hexagonal II phase. Its structure has been characterized primarily by X-ray diffraction and freeze-fracture electron microscopy. In \textsuperscript{31}P NMR spectra, cubic phase behaves as an isotropic phase. A schematic representation of the structure of cubic phase appears in Fig. 4.15.

7. Subphase for Phospholipid Bilayers

At temperatures near 0°C, and on extended incubation, some phosphatidylcholines can enter a subphase. The kinetics of entering this phase are slow, much slower than for the main gel to liquid crystalline transition. This phase exhibits properties similar to that observed in a dehydrated solid state. Instead of slow axial rotation, apparently little or no phospholipid axial rotation takes place, nor is there any significant lateral diffusion. This phase, often an $L_{	ext{c}}$ phase, is dehydrated relative to the hydrated gel state, $L_{	ext{g}}$.

8. Solution Phase

In addition to forming the wide variety of phase structures described above, phospholipids can also exist in a solubilized form. Solubilization most readily occurs in organic solvent. For example, in methanol, phospholipids occur as monomers, dimers, and trimers in solution. However, in chloroform, particularly wet chloroform, the phospholipids inhabit inverted micelles. Any water in the system is trapped next to the headgroups in the center of the inverted micelle.

In addition, phospholipids can exist in solution in an aqueous phase. For more familiar phospholipids with long-chain fatty acids, the solubility in the aqueous phase is vanishingly small. However, dipalmitoylphosphatidylcholine, because of its short chains, can exist as a monomer in the aqueous phase and has a CMC of about 10 mM. Apparently, the unfavorable entropy terms arising from contact between the solvent and the hydrocarbon chains are more than compensated by favorable terms due to the interaction of the polar headgroups with the water.

Cholesterol also is best solubilized in organic solvent, although it can self-associate even in nonaqueous solvents. Its solubility is small in the aqueous phase. However, the aqueous solubility of cholesterol is significant enough to play a role in the transfer of cholesterol from one membrane to another.

B. Phase Transitions

1. Gel to Liquid Crystalline Phase Transition

This discussion will now return to the structure of the lipid bilayer. The lipid bilayer represents the dominant form of assembly for lipids in cell membranes. Some of the unique properties of the phospholipid bilayer are bestowed on biological membranes.

There is some order to the structure of membranes; that is, there are limitations on the freedom of motion of molecules in the membranes. Because of the hydrophobic effect, the lipids of the bilayer cannot translate extensively in a direction normal to the bilayer surface. In other words, the lipids are not inclined to pop out of the membrane. However, the lipids can translate with considerable freedom and rapidity in the plane of the membrane, parallel to the membrane surface. Hence, to a first approximation the lipids in the membrane constitute a two-dimensional fluid. But the phospholipid bilayer is not a true liquid. Under most physiological conditions, the bilayer is in a liquid crystalline state ($L_{	ext{c}}$) as opposed to a true solid or a true liquid. Consideration of the properties of a synthetic phosphatidylcholine will help illuminate this concept.

Dipalmitoylphosphatidylcholine (DPPC) is quite different in its fatty acid composition from most natural phosphatidylcholines (although it can be found in biological membranes as a rare species and in pulmonary surfactant as the major species). Instead of having a saturated hydrocarbon chain at position 1 and an unsaturated hydrocarbon chain at position 2, it contains palmitic acid (16:0) at both positions. When this phospholipid is hydrated it forms multilamellar liposomes, just like other phosphatidylcholines and several other classes of phospholipids.

Above 42°C, these model membranes are in a liquid crystalline state, $L_{	ext{c}}$. Laterally, in the plane of the membrane, the phospholipid bilayer is disordered, because of two-dimensional lateral diffusion of the phospholipids. Nevertheless, phospholipid movement is inhibited by some organizational constraints as described above. The phospholipids are therefore not in a true liquid state in which their movement would be isotropic (in other words movement in any direction would be equally likely). Neither are these phospholipids in a solid state. This liquid crystal state of the phospholipids is therefore distinguished from the solid and the liquid state. A liquid crystal retains at least one dimension of order relative to the solid state.
growth temperature, a large fraction of the membrane lipids are in the gel state for many of the cases measured. However, in no case when the membrane is fully gel is growth supported. Apparently some liquid crystalline domains are required in the membrane for proper biological function.

Some natural membranes, including microsomal membranes, may also exhibit a gel to liquid crystal phase transition, though not normally in the region of physiological temperature. Furthermore, there may be microscopic domains of lipid in biological membranes that undergo phase transitions near physiological temperatures. Sphingomyelin is a commonly occurring lipid in biological membranes (for example, plasma membranes) that, when isolated in pure form, undergoes a gel to liquid crystalline phase transition near physiological temperature. Thus speculation exists on the presence of sphingomyelin-rich domains in plasma membranes that may be in the gel state under certain conditions. Much remains to be elucidated.²²

2. LAMELLAR TO HEXAGONAL II PHASE TRANSITION

The transition from the lamellar phase to the hexagonal phase can be detected by a variety of methods. X-ray diffraction shows a distinct change in the spacing of the reflections: 1 : 2 : 3 (lamellar) to 1 : 3 : 2 : 5 (HII). Freeze-fracture electron microscopy reveals the development of tube-like structures characteristic of that phase. ³¹P nuclear magnetic resonance (NMR) shows pronounced changes in the spectral shape (³¹P powder pattern) arising from the phospholipids due to the tubular shape of the HII phase (see Fig. 4.7). Calorimetry does not sense as distinctive a change as it does in the case of a gel to liquid crystalline phase transition. An endotherm is observed, but of less magnitude than observed in the gel to liquid crystalline phase transition. Therefore the lamellar to hexagonal phase transition is different in its properties from the gel to liquid crystalline phase transition.

Table 4.3 offers some examples of midpoint temperatures, Th, for La to Hn phase transitions of some lipids. Some of the influences on this phase transition temperature, such as unsaturation, are evident in Table 4.3.

Another important observation regarding the La to Hn phase transition is that the kinetics of the phase change can be much slower than the gel to liquid crystalline phase transition. Particularly notable is that the reversibility of this phase change (Hn to La) is often not demonstrable on a time scale of seconds or minutes. The kinetics of the latter transition are in fact even slower than the La to Hn phase transition, creating considerable hysteresis in measurements of this phase transition. Commonly, the La to Hn phase transition occurs rapidly on a time scale of minutes or faster during the heating of the sample. In contrast the Hn to La phase transition often requires prolonged incubation (hours or days) at temperatures below the transition temperature. Repeated cycling of the sample through Th can convert the system to the cubic phase.²⁶

These observations suggest unusual kinetics and pathways for this transition. In fact, it is not clear what pathway this transition follows. Some investigators suggest that the transition involves an interlamellar attachment that has geometric similarities with cubic phase.²⁷ Figure 4.15 shows one model of an intermediate between the La and the Hn phase. The kinetics of the pathway followed appears to depend on the lipid and ion

<table>
<thead>
<tr>
<th>Species of PE</th>
<th>Th (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0, 16:0</td>
<td>123</td>
</tr>
<tr>
<td>16:0, 16:1</td>
<td>71</td>
</tr>
<tr>
<td>18:0, 18:1</td>
<td>10</td>
</tr>
<tr>
<td>18:1, 18:1 monomethyl</td>
<td>73</td>
</tr>
</tbody>
</table>