

Chapter 2

The Structures and Properties of Membrane Lipids

The previous chapter briefly described the historical development of the concept that the lipid bilayer forms the structural basis of all biomembranes. It is important to understand the detailed structure of the lipid bilayer as well as the thermodynamic principles underlying its stability in order to approach an understanding of biological membranes. In addition, some phospholipids spontaneously organize in structures which are not bilayers, such as the inverted hexagonal H_{II} phase, and these lipids have been postulated to play specialized roles within membranes (see 303,267). In this chapter we will survey the structures and thermodynamics of lipid–water systems, with the emphasis on those features which give some insight into the properties of biological membranes.

2.1 Lipid Crystals (see 6 for review)

High-resolution structures from X-ray crystallography have been reported for several membrane lipids: lysophosphatidylcholine (605), dimyristoyl phosphatidic acid (594), dimyristoyl phosphatidylcholine (1133), dilauroyl phosphatidylethanolamine (636), dimyristoyl phosphatidylglycerol (1126), and a cerebroside (1125). These crystals contain very little water. However, it appears that the lipid structures in these crystals are similar to those adopted in the fully hydrated forms. Figure 2.1 shows the conformations adopted by some of these membrane lipids within the crystals. The glycerol carbon atoms, and the corresponding atoms in sphingosine, have been blackened for clarity. Figure 2.2 shows some of the structural notation used to describe the lipid conformation.

Let us start by considering the crystal structure of dilauroyl phosphatidylethanolamine (Figure 2.1A). The most salient features of these crystals are

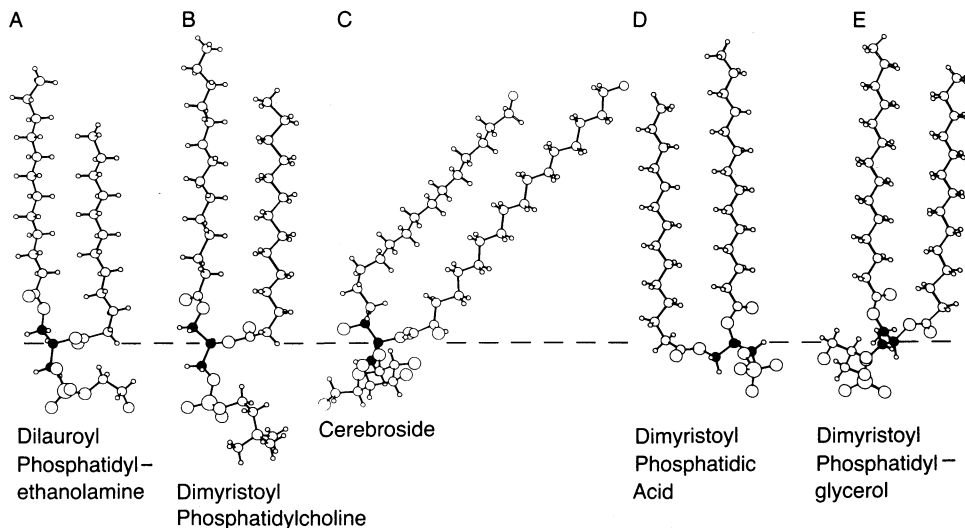


Figure 2.1. Structures of five membrane lipids as determined by X-ray crystallography. The three glycerol and sphingosine carbon backbone atoms have been colored black. Note A, B, and C have a similar conformation for the glycerol or sphingosine moiety, whereas D and E are each different. Adapted from references 604 and 636 (A); 1133 (B); 1125 (C), 594 (D) and 1126 (E). Figures kindly provided by Dr. I. Pascher.

1. The molecular area (S , as in Figure 2.2) is 39 \AA^2 .
2. The polar headgroup is virtually parallel to the plane of the bilayer. In fact, the amino group is hydrogen bonded to the unesterified phosphate oxygens of an adjacent molecule. The glycerol group is essentially oriented normal to the plane of the bilayer.
3. The *sn*-2 fatty acid chain extends parallel to the plane of the bilayer for the first two carbons and then is directed down into the bilayer. The *sn*-1 fatty acid extends directly into the bilayer.
4. The acyl chains are directed perpendicular to the bilayer surface and, with the exception of the initial part of the *sn*-2 fatty acid, are in a fully extended, i.e., all-*trans*, configuration.

While similar in many respects, the crystal structures of the phosphatidylcholine and the cerebroside have important differences from that of phosphatidylethanolamine. The most significant and obvious difference is the tilt of the acyl chains, most evident in the structure of the cerebroside. This is rationalized as being due to a simple packing problem. The bulky polar headgroups in these lipids do not allow the relatively simple structure seen with dilauroyl phosphatidylethanolamine. The cross-sectional areas (S) required by these headgroups are larger than the 39 \AA^2 occupied by the acyl chains of each molecule ($2\sum$, as in Figure 2.2). In the case of the cerebroside, the packing problem is eliminated by the tilting of the acyl chains with respect to the bilayer normal. This effectively increases

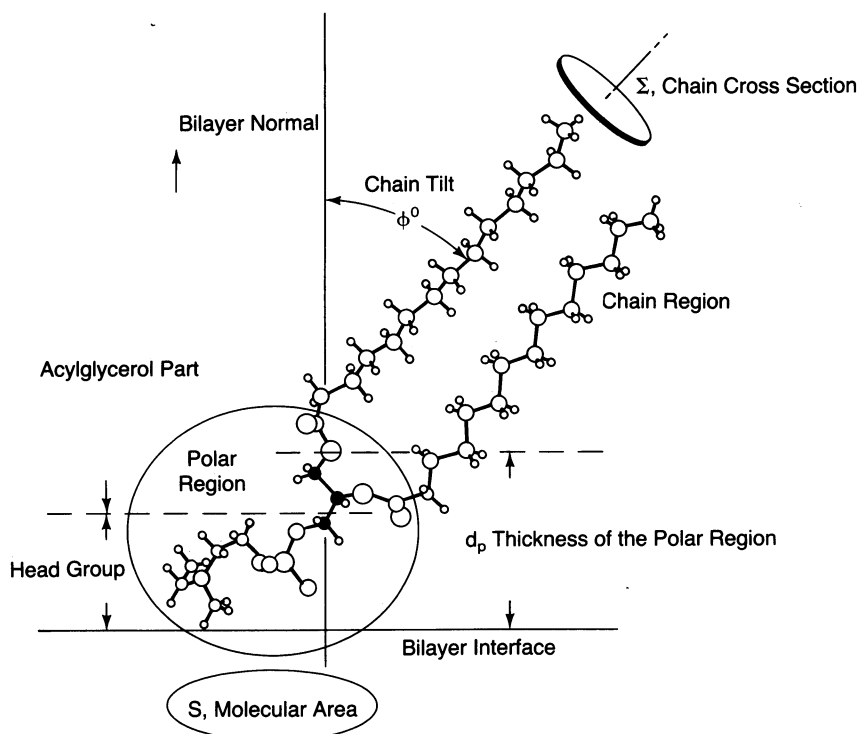
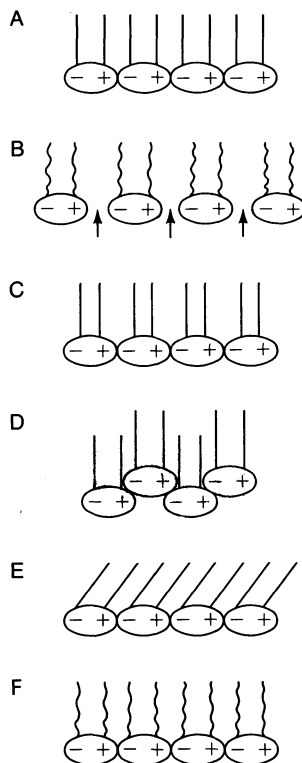


Figure 2.2. Schematic of a phosphatidylcholine molecule showing the structural notation defining various regions. Note that the cross-sectional area of the hydrocarbon chain, Σ , is taken perpendicular to the chain axis. The cross-sectional area parallel to the plane of the membrane will be, in this case, significantly larger due to the tilt of the chains. Adapted from ref. 604.

the cross-sectional area of the acyl chains with respect to the plane of the bilayer. Figure 2.3 shows schematically how the chain tilt allows for stabilizing interactions between the acyl chains of adjacent molecules, while maintaining the large molecular area due to the bulky headgroup. The acyl chains in the dimyristoyl phosphatidylcholine are tilted only about 12° away from the bilayer normal, in contrast to 41° in the cerebroside (1133). The diacyl phosphatidylcholine solves the problem of packing the bulky headgroups by having adjacent molecules alternately displaced along the bilayer normal, schematically shown in Figure 2.3D. There is substantial evidence that, in some cases, fully hydrated lipid bilayers in the gel phase also have substantial chain tilting (e.g., see 604, 1320; Section 2.2). This is one example where simple steric arguments, i.e., taking into account lipid “shapes” (S vs. 2Σ), are very useful in rationalizing lipid structures. Further examples will be described in Section 2.34.

In all the crystal structures, except that of phosphatidic acid, the initial part of the *sn*-2 fatty acyl chain is directed parallel to the bilayer surface. This feature

Figure 2.3. Schematic showing packing adaptations to differences in the cross-sectional areas of the polar group and the acyl chains (S and Σ , in Figure 2.2). These figures depict the behavior of phosphatidylethanolamine and phosphatidylcholine. (A) Phosphatidylethanolamine in a crystal, where the space requirements of the headgroup and the two acyl chains are the same. (B) Phosphatidylethanolamine in a liquid crystalline lamellar phase, where the area per molecule requires that the headgroup lattice be disrupted. This will occur only if water or other polar molecules can bridge the gap between lipid neighbors and stabilize the structure. (C) Phosphatidylcholine in a hypothetical arrangement illustrating that the space required by the headgroup (50 \AA^2) is substantially larger than required by the acyl chains (38 \AA^2). This requires some packing adjustment. (D) One adjustment of phosphatidylcholine observed in crystals is for the headgroups to be displaced in an overlapping fashion. (E) An adjustment observed in the lamellar gel phase of phosphatidylcholine is for the chains to be tilted such that they accommodate a larger cross-sectional area (50 \AA^2). (F) In the lamellar liquid crystalline phase, the size of the headgroup of phosphatidylcholine does not require the headgroup lattice to be disrupted. Adapted from ref. 604.



has also been confirmed by nuclear magnetic resonance (NMR) of phosphatidylethanolamine and phosphatidylcholine bilayers and for the phospholipids in membranes from *E. coli* (487). The physiological relevance of this feature is not known. However, it has been noted that in egg phosphatidylcholine, the average length of the *sn*-2 fatty acid is 18, whereas the average length of the *sn*-1 fatty acid is 16 carbons. This may be in compensation for the configuration of the 2-fatty acid, so that the two acyl chains extend about the same depth from the bilayer surface.

In summary, there are five major features of the crystal structures which have importance for considering the structure of the lipid bilayers:

1. The structures observed are all lamellar, with the polar and nonpolar groups organized as in a bilayer.
2. There are packing problems resulting from having a bulky headgroup such as in phosphatidylcholines and cerebroside ($S > 2\Sigma$). Such packing considerations are important determinants of the properties of membrane lipids, not only in crystals but also in model membranes and, possibly, within biomembranes.
3. The polar headgroups essentially lie flat in the plane of the bilayer, with intermolecular hydrogen bonding where possible.
4. Acyl chains (saturated) are in an all-*trans* configuration.

5. In most cases, the *sn*-2 fatty acyl chain does not extend into the bilayer until after the C-2 position.

Each of these features has been confirmed for lamellar structures formed by lipid-water mixtures for the gel phase (points 1, 2, 3, 4, 5) and/or the liquid crystalline phase (points 1, 3, 5) (see next section). The structures of lipids in crystals are an excellent starting point for understanding the conformations of lipids within biological membranes.

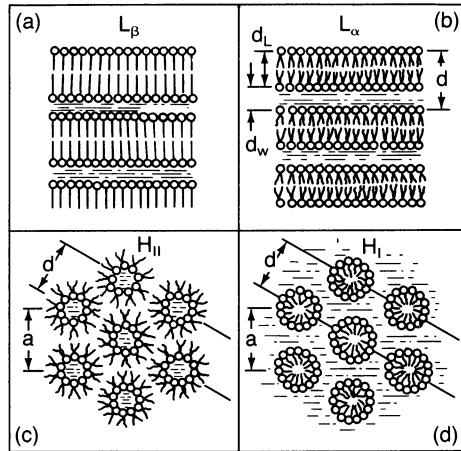
2.2 Lipid–Water Mixtures

Mixture of lipids and water are polymorphic. Even for single purified lipids there is more than one kind of organized structure when hydrated. The particular form which predominates depends on such parameters as the lipid concentration, temperature, pressure, ionic strength, and pH. X-ray diffraction techniques have been particularly valuable in defining the kinds of structures in lipid–water systems, and this is most often studied as a function of lipid concentration and temperature, with the data presented in the form of a phase diagram indicating the structure in various regions of the “temperature–concentration” plot. Differential scanning calorimetry is often used along with X-ray diffraction to define the phase boundaries of the lipid–water phase diagrams. These studies are usually performed at high lipid concentrations [$>40\%$ lipid (w/w)]; however, many of the structures which have been characterized at high lipid concentrations also exist in lipid dispersions in a large excess of water.

The major organized forms of lipid–water systems (see 1334, 263 for reviews) are depicted schematically in Figure 2.4. The major forms are

1. *Lamellar liquid crystalline phase* (L_α): This form is what is usually thought of as representing the bulk of the lipids in the biological membrane. There is two-dimensional order, but there is considerable disorder in the acyl chains, as indicated by the X-ray diffraction data.
2. *Lamellar gel phase* (L_β): This is formed at low temperatures in those lipids which form the lamellar structure. The molecules are packed more tightly together (smaller surface area per molecule) and the acyl chains are much more highly ordered, corresponding to the all-trans configuration found in the structure of lipid crystals. Because the chains are maximally extended in the gel phase, the bilayer thickness is greater than in the liquid crystalline phase. The density of the gel phase is slightly greater than that of the liquid crystalline phase. In lipids which have bulky polar headgroups, such as dipalmitoyl phosphatidylcholine, the acyl chains are tilted with respect to the bilayer normal (700, 461) similar to structures seen in some lipid crystals (Figure 2.1). The chain tilt is often denoted by a prime (L'_β). It is interesting to note that dispersions of phosphatidylcholine in solvents containing some alcohols (1346) or glycerol (1084) form an unusual gel phase in which the acyl chains from opposing halves of the “bilayer” are fully interdigitated. The biological significance of this is not known.

Figure 2.4. Schematic representations of lipid–water phases: (a) lamellar gel; (b) lamellar liquid crystalline; (c) hexagonal type II; (d) hexagonal type I. Various dimensions that can be measured by X-ray diffraction are indicated. Adapted from ref. 1334.



3. *Hexagonal I phase (H_I)*: In this form, the lipids are organized in the form of cylinders with the polar groups on the outside, in contact with water. The cylinders are packed in a hexagonal pattern.
4. *Hexagonal II phase (H_{II})*: The lipids are in the form of cylinders, but in this case the polar groups face the inside, where there is a column of water. Again, the cylinders are packed in a hexagonal array.

One major point is that a number of lipids form nonbilayer structures. In fact, many purified membrane lipids do not form stable bilayers, but prefer the H_{II} hexagonal phase. Examples are unsaturated phosphatidylethanolamines and the glycolipid monogalactosyldiglyceride (see Figure 2.5). The reasons for this, and the possible biological relevance, is discussed in subsequent sections.

2.21 Lipid Hydration

X-ray diffraction methods (see 1320, 910) can yield the dimensions indicated in Figure 2.4. As a general rule, the dimension determined primarily by the length of the acyl chains remains nearly constant as the percent water in the system is increased. The lipid headgroups bind to water and become hydrated. This has been studied extensively using NMR techniques, either $^1\text{H-NMR}$ or $^2\text{H-NMR}$. Results with phosphatidylglycerol, phosphatidylcholine, and phosphatidylethanolamine using $^2\text{H-NMR}$ indicate a primary hydration shell of 11–16 water molecules per lipid, in rapid exchange with the bulk water (123). Other measurements indicate that the phosphatidylethanolamine headgroup binds fewer water molecules than the headgroup of phosphatidylcholine, and it has been speculated that this lower hydration favors the formation of the nonlamellar hexagonal H_{II} phase by unsaturated phosphatidylethanolamines (1616).

Many lipids swell in water. Lipids which are neutral or isoelectric (e.g., phosphatidylcholine) show no or limited swelling, as indicated by the limited thickness of the water layer between lamellae (e.g., see 195, 600). In excess water,

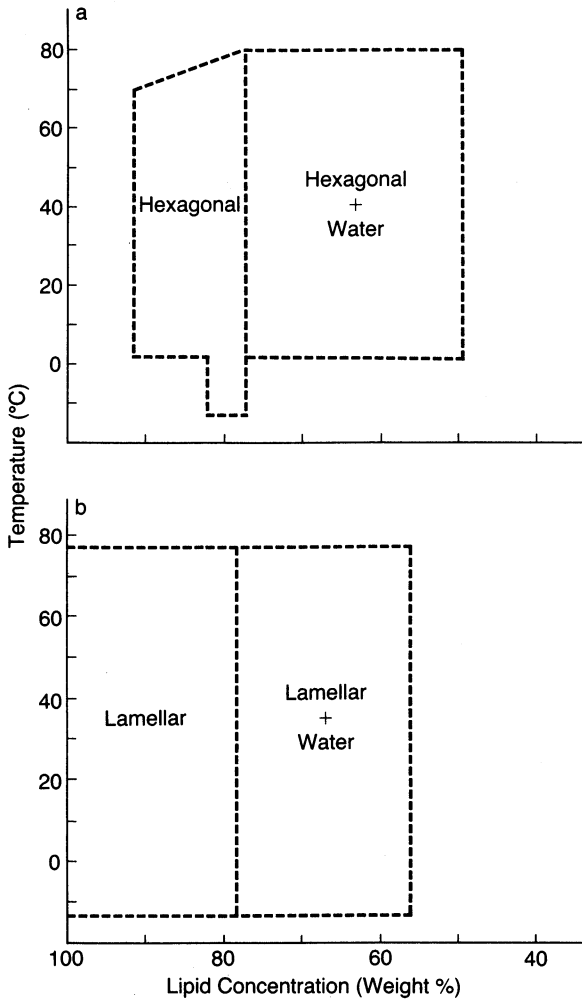


Figure 2.5. Phase diagrams of (a) monogalactosyl diacylglyceride (MGDG) and (b) digalactosyl diacylglyceride (DGDG) extracted from leaves. The acyl chains in both cases are predominantly unsaturated. Adapted from ref. 1334a.

two phases exist, a multilamellar lipid structure in equilibrium with bulk water. Charged lipids show continuous swelling with water added between lamellae up to a certain threshold at which point two phases form, a fully hydrated unilamellar vesicle with bulk water (600). The swelling and relative stability of the multilamellar and unilamellar forms are determined by electrostatic interactions. Low ionic strength results in destabilizing the multilamellar forms. Lipid mixtures with only a few percent of a charged lipid can exhibit continuous swelling behavior.

The polarization of water molecules by the lipid polar headgroups results in

a strong repulsive interaction when two bilayers are brought close together (see 1193, 195). This “hydration” force keeps hydrated bilayers at least 30 Å apart. It is this hydration force which provides the major energetic barrier which must be overcome to obtain membrane fusion (see Chapter 9). Phosphatidylethanolamine vesicles tend to aggregate, possibly because the hydration of this headgroup is relatively low (see Section 9.51).

Studies using electron spin resonance (ESR) probes designed to monitor the polarity at different distances from the bilayer surface indicate that water is able to partially penetrate into the hydrocarbon core in the liquid crystalline phase (551). Studies using neutron scattering show that water does not extend beyond the glycerol backbone of the polar headgroup in bilayers in the gel phase (1606).

2.22 Examples of Phase Diagrams of Single Component Lipid–Water Systems

Figure 2.5 shows the temperature–concentration phase diagrams of two common glycolipids isolated from plants (see 1334, 1041, 533). Digalactosyl diglyceride (DGDG) forms a stable lamellar phase. Only a single hydrated phase is present at low water content. Beyond about 20% water, two phases are present, a multilamellar hydrated lipid phase and bulk water. This lipid does not exhibit continuous swelling.

Box 2.1 Some Lipids Isolated from Biological Membranes Do Not Form a Stable Bilayer

The phase diagram of monogalactosyl diglyceride (MGDG) is also shown in Figure 2.5. Note that the removal of a single sugar residue from the lipid headgroup has a drastic effect on the structure of the lipid–water system. Only hexagonal phase H_{II} is formed, and the lipid does not form a stable bilayer by itself. This highly unsaturated galactolipid makes up about 20% of the dry weight of the chloroplast thylakoid membrane and about half of the lipid in the membrane (533). It has been suggested (1041, 533) that MGDG plays an important role in stabilizing the regions of high concave curvature in the thylakoid membrane system and may possibly play other specialized roles.

Another example is provided by phosphatidylethanolamine isolated from the bacterium *Pseudomonas fluorescens*. This lipid is about 75% of the total phospholipid of the membrane (262). It has a heterogeneous and unsaturated acyl chain composition and forms a stable hexagonal H_{II} phase in excess water at room temperature (1334, 262). Clearly, the presence of other components in the membrane stabilizes this lipid in the bilayer form. Saturated phosphatidylethanolamines also show complex phase behavior but do not form H_{II} phase near physiological temperatures (1315). Changes in phase that occur as a function of water composition at constant temperatures are called *lyotropic transitions*.

Figure 2.6 shows a phase diagram of dipalmitoyl phosphatidylcholine (see 1269). This lipid exists in a lamellar form under most conditions. It “swells” upon addition of water until a maximum amount of water is absorbed between the bilayers, at which point a two-phase system with multilamellar liposomes is formed. Note that there is a distinct phase in between the gel (L_{β}) and liquid crystalline (L_{α}) lamellar forms. This is the so-called “ripple phase” (P_{β}). In this phase, the surface of the bilayer is rippled and presents a wave-like appearance in electron micrographs (Figure 2.7). The thermotropic phase transition $P_{\beta} \rightarrow L_{\alpha}$ is termed the main transition, whereas the transition $L_{\beta} \rightarrow P_{\beta}$ is called the pre-transition (see Section 2.4).

Lipids that have a bulky polar headgroup, such as choline, generally show the pre-transition, indicating a phase which is intermediate between the gel and liquid crystalline phases (604). In dipalmitoyl phosphatidylcholine the acyl chains are probably tilted in the P_{β} phase (461), but Raman studies (181) indicate that the chains are still predominantly all-*trans*, as in the gel phase (see Section 2.4).

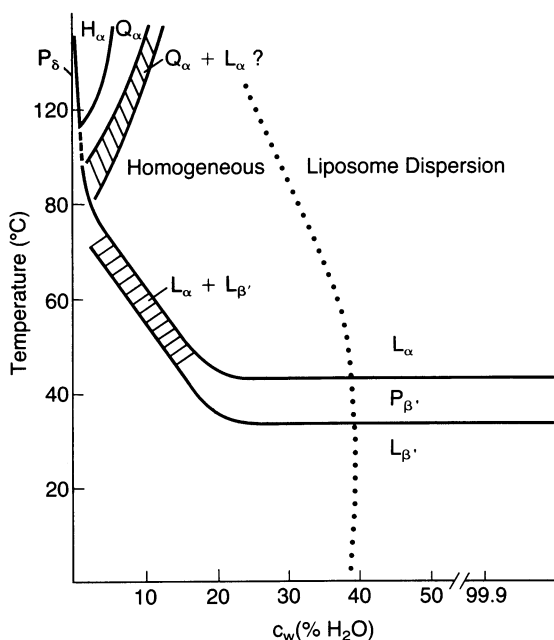


Figure 2.6. Phase diagram of dipalmitoyl phosphatidylcholine in water. The percentage of water is indicated on the abscissa with pure lipid on the left and a dilute lipid suspension on the right. Indicated are the lamellar gel phase (L_{β}), lamellar liquid crystalline phase (L_{α}), and intermediate ripple phase (P_{β}). At high temperatures and low hydration, other phases can form (Q_{α} , cubic phase and H_{α} , hexagonal phase). The dotted line indicates the maximum absorption of water by the homogeneous lipid-water mixture. Adapted from ref. 1269.

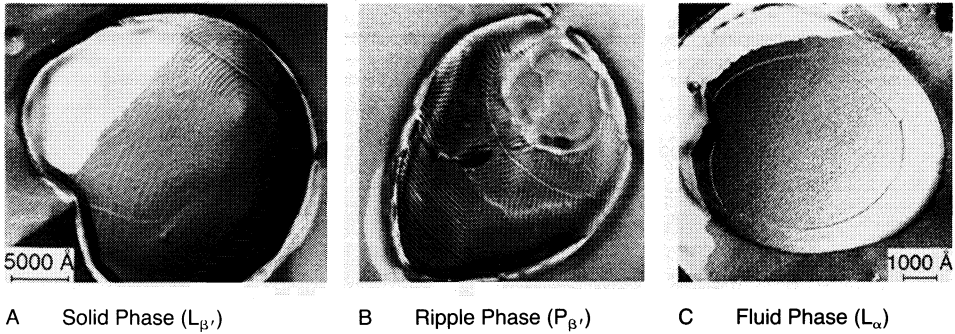


Figure 2.7. Freeze-etch electron micrographs showing the textured appearance of gel (L_{β}), ripple (P_{β}), and liquid crystalline (L_{α}) phase of pure phosphatidylcholines. (A) Dipalmitoyl phosphatidylcholine at 25°C; (B) dipalmitoyl phosphatidylcholine at 35°C; (C) dimyristoyl phosphatidylcholine at 25°C. Adapted from ref. 1269. Photographs kindly provided by Dr. E. Sackmann.

2.23 Two Techniques Used for Examining Lipid Polymorphism

Besides X-ray diffraction, a number of techniques have been used to characterize the properties of lipid phases. One that has been particularly useful is freeze-fracture electron microscopy. A second technique, ^{31}P -NMR, has been used more recently, with particular application to detecting nonbilayer structures, which are felt by some to play specialized roles in biomembranes (e.g., 1507, 264).

(1) Electron Microscopy of Freeze-Etched Lipids. One of the techniques which has been very useful in examining the architecture of the various lipid phases is freeze-etch electron microscopy (see Chapter 1 and refs. 263, 313, 1509, 783). Examples are shown in Figure 2.7. The liquid crystalline phase (L_{α}) always presents a smooth surface, whereas the P_{β} phase appears rippled. The gel phase (L_{β}) can appear smooth or, depending on the manner of sample preparation, can exhibit a spiral pattern resulting from temporary defects in the close-packed structure. The hexagonal phase (H_{II}) formed by lipids such as unsaturated phosphatidylethanolamines can also be identified by freeze-etch electron microscopy (Figure 2.8), looking like a stack of cylinders. In preparing samples, the lipids are equilibrated at the appropriate condition to form a particular structure and then rapidly frozen before the lipids can rearrange.

Finally, electron microscopy of lipid bilayers has been used to examine “lipidic particles” (Figure 2.9). These are frequently observed in binary mixtures of lipids where one lipid prefers H_{II} and the other a bilayer configuration (see 263, 1507, 1508). These particles are observed in pure lipid preparations and are different from the “particles” associated with proteins which are observed in biomembranes and in reconstituted protein–lipid systems (see Figure 1.6). It has been speculated that lipidic particles represent regions of inverted micelles within the bilayer and

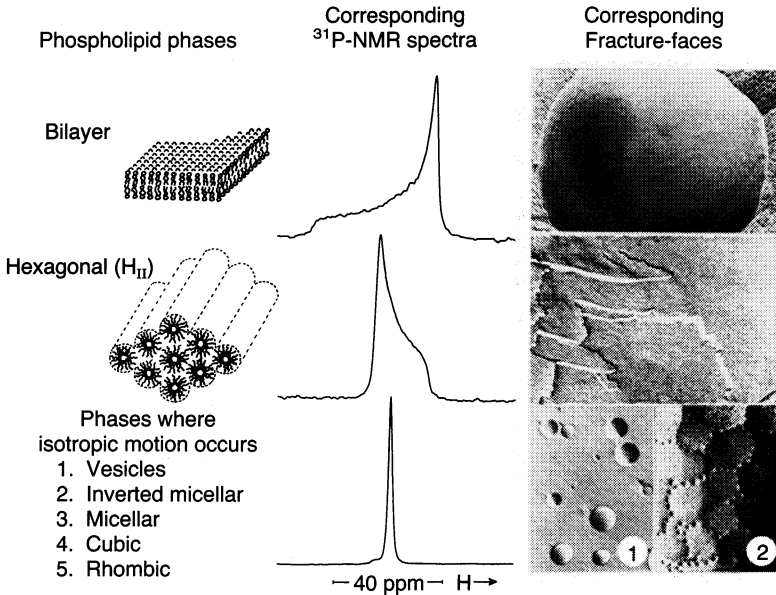


Figure 2.8. ^{31}P -NMR and freeze-fracture characteristics of phospholipids in various phases. Notice that the lamellar liquid crystalline phase (L_{α}) and hexagonal H_{II} phase have very different and diagnostic ^{31}P -NMR spectra as well as quite different appearances in freeze-fracture electron micrographs. The bilayer spectrum was obtained with egg yolk phosphatidylcholine and the hexagonal phase spectrum was obtained with phosphatidylethanolamine prepared from soya beans. The sharp ^{31}P -NMR spectrum, characteristic of rapid motion, is most often observed from either small unilamellar vesicles (photo 1) or large lipid structures containing "lipidic particles" (photo 2). Adapted from ref. 263. Photographs kindly provided by Dr. P. Cullis and Dr. M. Hope.

that they play a function in biological processes such as facilitating membrane fusion or stabilizing regions of high curvature, as in the thylakoid membrane (263, 1507, 1041, 533). However, solid evidence concerning any biological significance of lipidic particles is, so far, lacking.

(2) ^{31}P -NMR of bilayers (see 1454). This technique is also used to characterize the structural properties of hydrated lipids (see 263, 1318, 264, 266, 487, 748). For example, phospholipids in the H_{II} hexagonal phase give rise to a distinctly different ^{31}P -NMR spectrum than do phospholipids which are in a lamellar phase (266, 487) (Figure 2.8). This method has been used to detect lamellar $\rightarrow H_{II}$ transitions in lipids and lipid mixtures (e.g., 487, 748). This technique is subject to ambiguities, especially when interpreting spectra which indicate "isotropic" averaging caused by relatively rapid motions. One structure presumed to be consistent with such a spectrum is the "lipidic particle." However, this is not a unique interpretation of such a spectrum. ^{31}P -NMR has been shown to be reliable in detecting the presence of hexagonal H_{II} phase in pure lipid dispersions, but

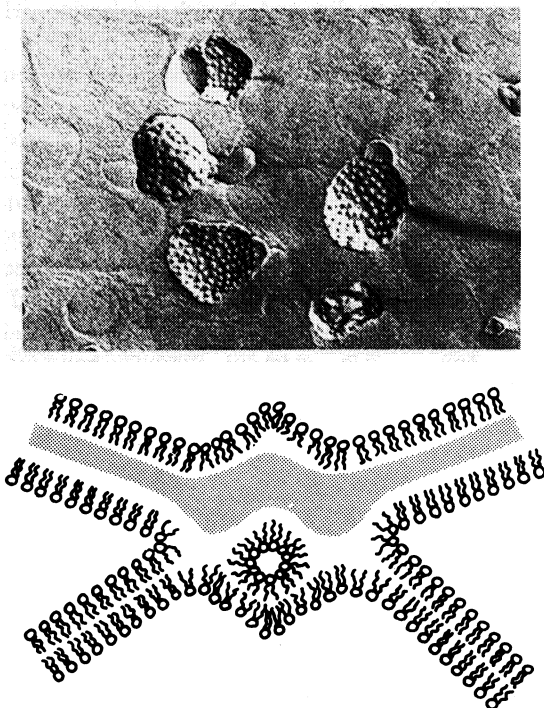


Figure 2.9. Freeze-fracture micrograph of lipidic particles induced by Ca^{2+} in mixture of soya phosphatidylethanolamine and cardiolipin (4:1 molar ratio). A hypothetical model depicting a lipidic particle as an inverted micelle is shown below the micrograph. The shaded region indicates the fracture region. From ref. 263. Figure kindly provided by Dr. P. Cullis and Dr. M. Hope.

such interpretations must be viewed with caution when applied to biomembranes, unless other techniques are also used.

^{31}P -NMR has also been used to study the orientation and dynamics of the phospholipid polar headgroups, as well as the perturbations caused by integral membrane proteins on the bilayer (see next section and Chapter 5).

2.24 Lipid Headgroup Orientation in the Bilayer (for reviews, see 604, 1320)

A number of techniques indicate that in lamellar phospholipid-water dispersions, the lipid polar headgroups are oriented approximately parallel to the plane of the bilayer, as observed in lipid crystals (Section 2.1). For phosphatidylcholines, this has been shown for both the gel and liquid crystalline phases by X-ray and by neutron diffraction. ^2H -NMR studies are also consistent with this orientation, but cannot rule out alternate interpretations. Results indicate similar structures for

phosphatidylglycerol, sphingomyelin, and phosphatidylserine. ^2H -NMR studies on intact mouse fibroblast cells as well as on isolated membranes indicate that both the phosphatidylcholine and phosphatidylethanolamine components have their headgroups oriented parallel to the membrane surface (1293). In contrast, neutron diffraction studies show that phosphatidylglycerol isolated from *E. coli* has its headgroup oriented about 30° from the membrane surface, making its negatively charged phosphate moiety accessible for interaction with cations (995).

The orientation and dynamics of lipid headgroups may be influenced by intermolecular hydrogen bonds at the membrane surface (for review, see 115). Clearly, lipids such as phosphatidylserine, phosphatidylethanolamine, and various glycolipids can participate as donors and acceptors of hydrogen bonds. Studies on model membrane systems indicate headgroup hydrogen bonding can be important, even in the aqueous environment at the membrane surface, but the relevance to the structure of biomembranes is not known.

2.25 Acyl Chain Configuration and Packing in the Bilayer

Let us consider saturated chains first. There is free rotation about each C-C bond, with preferred energy minima, most easily seen in a Newman projection (Figure 2.10). The *trans* configuration is most stable and there is an estimated energy barrier of 3.5 kcal/mol to rotate past the eclipsed configuration to the *gauche* form. The all-*trans* configuration allows the chain to be maximally extended, whereas a *gauche* bond alters the direction of the chain. A sequence of *gauche-trans-gauche* for three consecutive C-C bonds results in a kink in the chain which effectively displaces the portions of the chain above and below the kink, as seen in Figure 2.11. Note that each *gauche* configuration can be designated g^+ or g^- depending

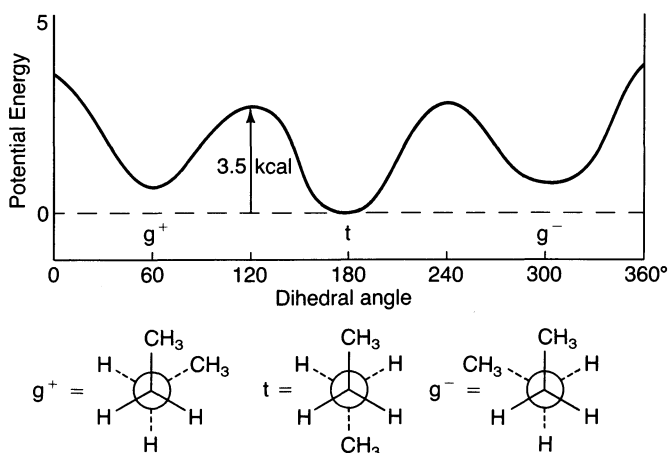


Figure 2.10. Potential energy curve for rotation about a carbon-carbon bond in an alkane. Below is the Newman projection diagram of the minimum energy *gauche* and *trans* conformations of butane: g^+ , g^- , and t . Adapted from ref. 535.

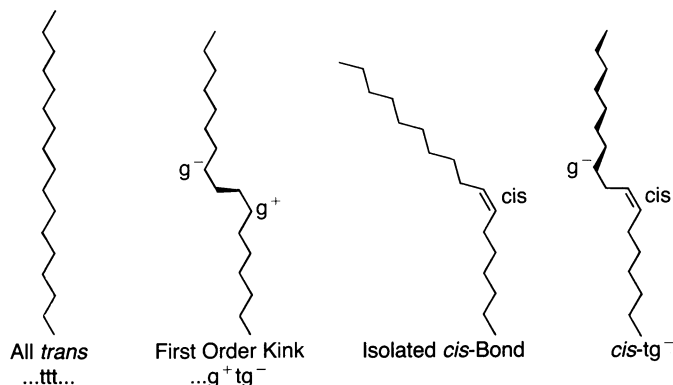


Figure 2.11. Illustration of several alkyl chain configurations. Adapted from ref. 808.

on the sense of rotation in going from C_1 to C_4 (see Figure 2.10). A kink which results in a simple displacement can be either g^+tg^- or g^-tg^+ . Almost all double bonds found in membrane lipids are *cis* and introduce the same kind of change of direction in the chain as does a *gauche* configuration. The presence of kinks, *cis*-double bonds, cyclopropyl groups (352), or other alterations from the simple all-*trans* chain configuration results in increasing the cross-sectional area of the hydrocarbon chain from the minimum of about 19 \AA^2 , and this has important consequences. The packing of lipids in the bilayer can be rationalized by considering the relative space requirements of the hydrocarbon chains and polar headgroup, using the same principles inferred from the lipid crystal structures (Section 2.1). These same principles will be discussed in Section 2.3 in predicting micelle shapes.

Numerous techniques including X-ray diffraction, neutron diffraction, and Raman and IR spectroscopies indicate that in the gel phase, the hydrocarbon chains of saturated diacyl phospholipids are predominantly in the all-*trans* configuration (see 604, 1320). The minimal cross-sectional area per molecule required by diacyl phospholipids is about 38 \AA^2 (2Σ in Figure 2.2). This is about the same as the area required by the phosphatidylethanolamine headgroup, and saturated phosphatidylethanolamines in the gel phase can pack with their acyl chains parallel to the bilayer normal as in the lipid crystals. However, as observed in crystals of phosphatidylcholines (605, 1133), the minimal packing requirement for the headgroup is in the range of 50 \AA^2 and, hence, dipalmitoyl phosphatidylcholine in the gel phase cannot pack in the same way as does phosphatidylethanolamine. In the gel phase, the acyl chains of dipalmitoyl phosphatidylcholine are tilted about 30° with respect to the bilayer normal, effectively increasing their cross-sectional area to be compatible with that of the headgroup; the chains remain in the all-*trans* configuration. In the liquid crystalline phase, the introduction of *gauche* configurations increases the effective chain cross section to at least 50 \AA^2 for the diacyl phospholipid, and in aqueous dispersions the effective liquid crystalline molecular area is typically in the range 60 to 70 \AA^2 (see 604). Hence,

in the liquid crystalline phase, the hydrocarbon chains are not tilted. The headgroups are sufficiently far apart under these conditions, and they require water or other polar molecules to act as spacers or to bridge between neighboring headgroups. ^2H -NMR indicates the thickness of the hydrocarbon domain of dipalmitoyl phosphatidylcholine is 35 Å in the liquid crystalline phase, compared to 45 Å expected if the chains were all-*trans* and oriented along the bilayer normal (see 1320). The shorter distance is due to *gauche* configurations (chain disorder), and the chains are basically aligned and perpendicular to the plane of the bilayer and are not coiled. Figure 2.12 illustrates the linear variation of bilayer thickness of fluid phase vesicles of diacyl phosphatidylcholines on the acyl chain length, determined by X-ray scattering (848).

2.26 Techniques Useful for Characterizing the Interior of the Bilayer

Two techniques which have been particularly useful for obtaining a detailed picture of the interior of the bilayer have been ^2H -NMR and vibrational spectroscopy (infrared and Raman spectroscopies). Proton and ^{13}C -NMR have been hindered in their application to membranes and lipid-water systems because of the demonstrated need to use ultrasonic dispersal to yield small vesicles or membrane fragments. The application of the technique of magic angle sample spinning eliminates this need, however, and this is likely to open the way for the application of state-of-the-art NMR methodologies to the study of model membranes and biological membranes (1093).

^2H -NMR and vibrational spectroscopy are nonperturbing and do not involve the use of probes embedded in the bilayer, which might influence the structure

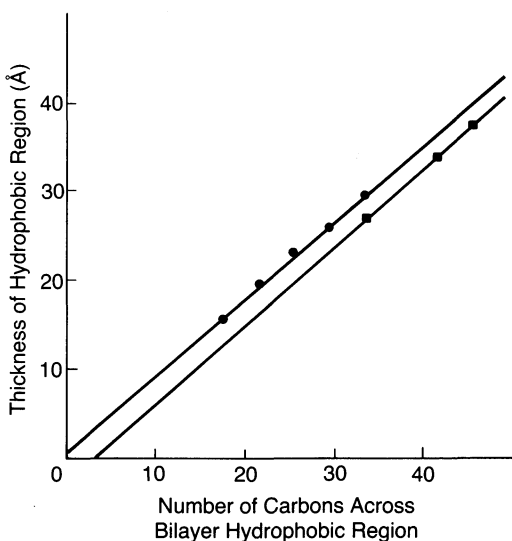


Figure 2.12. The measured thickness by X-ray scattering of the bilayer hydrocarbon region as a function of the number of carbon atoms in the hydrocarbon chain. The number of carbons is taken starting at C-2 of each acyl chain. Measurements were made on liquid crystalline diacyl phosphatidylcholines. Circles, saturated chains; squares, monounsaturated acyl chains. The molecular area was measured to be 65 to 70 Å². From ref. 848.

of the surrounding lipids. A brief description of these techniques and a summary of results relating to acyl chain configuration follow. The use of membrane probes for either ESR or fluorescence measurements will be discussed in Chapter 5 in connection with measurements of membrane dynamics and lipid-protein interactions.

Raman Spectroscopy

Laser Raman spectroscopy can be used to probe the physical state of model membranes and biomembranes (for reviews see 1513, 1544). The technique measures the energy differences between scattered photons and the incident photons caused by interaction with the vibrational modes of the sample. The vibrational modes and their intensities are very dependent on the physical state of the lipids, and the spectra of gel and liquid crystalline phospholipids are quite different (e.g., 477). Of particular use in monitoring these differences are the C-C stretch modes. For example, the appearance of *gauche* bonds at the expense of *trans* segments as lipid chains melt results in an increase in a band at ~ 1080 cm^{-1} . This technique indicates that some *gauche* bonds persist in the gel state until the temperature is decreased to very low temperatures, near -200°C . It is worth noting that the vibrational modes often involve the entire molecule, so quantitative interpretation in terms of particular conformations is not simple.

Typically, samples are about 1 mg/ml lipid suspensions. Membranes with chromophores (e.g., hemes) or impurities which result in fluorescence cannot be used. Background fluorescence makes measurement of the relatively small Raman signal impossible, and chromophores will cause heating problems, due to laser light absorption.

Infrared Spectroscopy (IR)

This technique also monitors the vibrational modes of the sample, but until recently the application of IR to biological samples was limited due to the inability to use aqueous suspensions. However, the recent development of Fourier transform infrared (FT-IR) instrumentation has eliminated many of these problems. Hence, lipid dispersions and biomembranes can now be examined by FT-IR (for reviews see 190, 32). This technique has the advantage over Raman spectroscopy of significantly improved sensitivity and the fact that fluorescence impurities or chromophores do not interfere with the measurement. As with Raman spectroscopy, the FT-IR spectrum is sensitive to changes in phase of polymorphic lipids. Hence, FT-IR has been used to monitor the pretransition in phosphatidylcholine bilayers (181), the main gel-to-liquid crystalline transition (180), as well as the transition from lamellar to hexagonal (H_{II}) exhibited by egg yolk phosphatidylethanolamine (919).

Changes in lipid chain conformation can be monitored by the frequency shifts of the CH_2 absorption bands, and these can be interpreted in terms of changes in the number of *gauche* isomers in the chains. For example, cholesterol causes the number of *gauche* configurations in dipalmitoyl phosphatidylcholine to decrease

above the main phase transition temperature (250), consistent with changes in the order parameter monitored by $^2\text{H-NMR}$ (1092). The incorporation of intrinsic membrane proteins in the bilayer (see Chapter 5), however, has quite a different effect, resulting in little or no change in the number of *gauche* isomers in the liquid crystalline phase but increasing the *gauche* isomers in the gel phase. The proteins interfere with the ability of the acyl chains to pack in the all-*trans* configuration (250).

$^2\text{H-NMR}$

By far the most detailed picture of the structure of the interior of the lipid bilayer has emerged from $^2\text{H-NMR}$ (for reviews see 1319, 291). Deuterium can be chemically substituted for hydrogen at specific places in the lipid molecule. This is a relatively benign substitution and is generally considered nonperturbing. The spectra of several deuterated derivatives of dimyristoyl phosphatidylcholine are shown in Figure 2.13. The separation between the two peaks is the quadrupolar splitting, $\Delta\nu_Q$, and this is dependent on the time-averaged orientation between the C-D bond vector and the bilayer normal (Figure 2.14). This time-averaged orientation is usually quantified in terms of an order parameter by the following equation:

$$S_{\text{CD}} = 1/2 (3\langle\cos^2\theta\rangle - 1)$$

where $\langle\cos^2\theta\rangle$ implies a time average and S_{CD} is the bond order parameter. Note that what is measured is the average of this value over all the molecules.

For $\theta = 0^\circ$, $\langle\cos^2\theta\rangle = 1$ and $S_{\text{CD}} = 1$

For $\theta = 90^\circ$, $\langle\cos^2\theta\rangle = 0$ and $S_{\text{CD}} = -1/2$

Random orientation, $\langle\cos^2\theta\rangle = 1/3$ and $S_{\text{CD}} = 0$

Frequently, the *molecular order parameter* (S_{mol}) is reported, which represents the orientation of the vector perpendicular to the plane formed by the CD_2 group (Figure 2.14). This indicates the average orientation of this segment of the acyl chain.

$$S_{\text{mol}} = -2S_{\text{CD}}$$

The order parameter obtained from $^2\text{H-NMR}$ reflects the average orientation and says little about the dynamics of the system or the range of motion.

Of critical importance is the fact that the local magnetic field sensed by a particular deuterium depends on the orientation of the C-D bond with respect to the external magnetic field. The motions due to molecular vibrations and rotations which influence the orientation of the C-D bond in the bilayer, in general, occur at rates sufficiently rapid ($>10^6 \text{ sec}^{-1}$) so that any particular deuterium senses a single average magnetic environment. The particular environment sensed depends on the surrounding atoms as well as on any constraints on the range of motion. This can be contrasted with Raman or infrared spectroscopies where *trans* and *gauche* rotamers interconvert at far slower rates (10^9 sec^{-1}) than the frequency

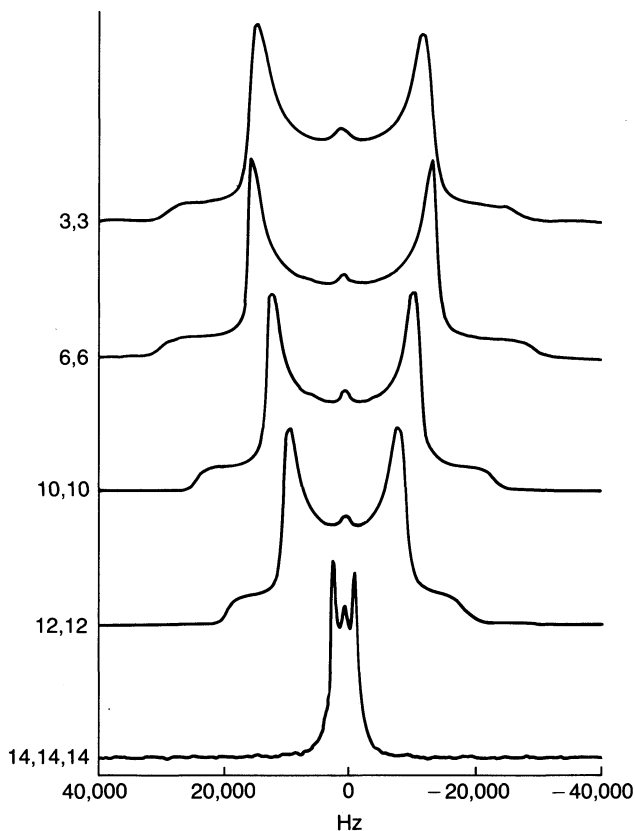


Figure 2.13. ^2H -NMR spectra of dimyristoyl phosphatidylcholine deuterated at different positions on the acyl chain. The numbers on the left indicate the position of the 2 (or 3) deuterium atoms in each chain. Note that the spectrum from the sample deuterated at the terminal methyl residue (14, 14, 14) is much more narrow, indicating considerable disorder in the middle of the bilayer. The figure was kindly provided by Dr. E. Oldfield.

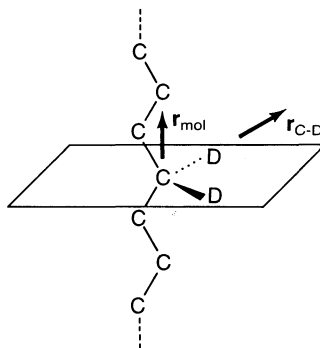


Figure 2.14. An illustration of the molecular vector (r_{mol}) and the C-D bond vector ($r_{\text{C-D}}$) which are used for computing order parameters obtained from ^2H -NMR.

difference separating the vibrational bands associated with each form ($\sim 10^{12}$ Hz). Hence, IR and Raman spectroscopies give what amounts to an instantaneous snapshot, summing spectroscopic contributions for *trans* and *gauche* rotamers. Other techniques, in particular ESR and fluorescence, are also used to obtain order parameters, and the use of these probe-dependent techniques will be discussed in Chapter 5.

Figure 2.15 shows the ^2H -NMR order parameters for a series of specifically deuterated derivatives of various phospholipids where the deuterium is present in specific methylene groups on the *sn*-1 palmitic acyl chain. The measurements are made on lipids or membranes in the liquid crystalline phase, since in the gel phase the spectra are very broad due to the rigidity of the lipids and are difficult to analyze. Two general remarks can be made about these results:

1. The order parameter is relatively constant for C-2 through about C-8 or C-10. The methylenes located toward the middle of the bilayer show considerably more disorder than those near the surface.
2. The same order parameter profile is obtained for synthetic lipids of various types, including phosphatidylcholine, phosphatidylserine, and sphingomyelin, and also for biological membranes which have incorporated deuterated probes. Hence, the profile is basically not sensitive to lipid chemical structure or membrane composition so long as the bilayer is in the liquid crystalline phase.

Quantitative interpretations of these data require molecular modeling using statistical mechanical techniques (e.g., 921; for review see 1161). For example,

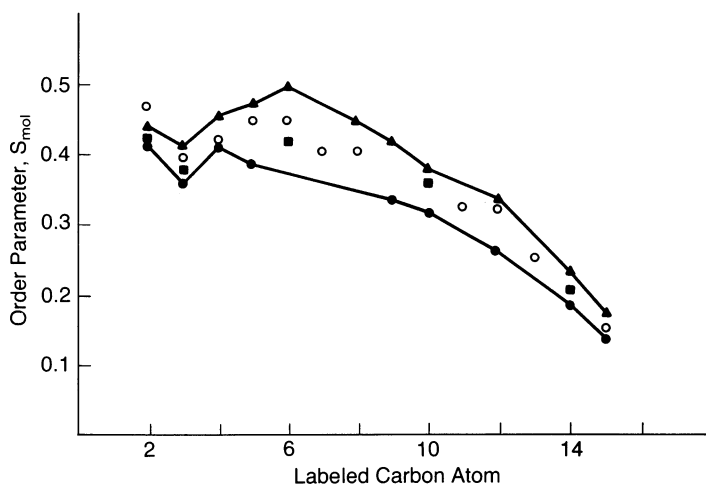


Figure 2.15. Normalized order profiles for different bilayers, showing the molecular order parameter as a function of position in the acyl chain. Closed circles, dipalmitoyl phosphatidylcholine; closed triangles, 1-palmitoyl-2-oleoyl phosphatidylcholine; closed squares, dipalmitoyl phosphatidylserine; open circles, *Acholeplasma laidlawii* membranes. Adapted from ref. 1321. Reprinted by permission of the publisher from "General Features of Phospholipid Conformation," by J. Seelig and J. L. Browning, FEBS Lett. 92, pp. 41-44, Copyright 1978 by Elsevier Science Publishing Co., Inc.

the data are consistent with the presence of about four or five *gauche* rotamers in each chain in dipalmitoyl phosphatidylcholine, but very few kinks (0.5/chain) (see 1320). Because each chain is essentially tethered at the bilayer surface, there is greater order in the portion of acyl chain nearest the surface. Note that the bilayer is obviously a highly cooperative system. An acyl chain cannot change direction without compensating changes in the neighboring chains. Hence, groups of adjacent chain segments must move in a cooperative manner. Deviations in chain segment direction from the bilayer normal will accumulate as one proceeds from bilayer surface to the interior. Hence, the disorder is maximal at the center of the bilayer, and in this region the chains appear similar to those in a paraffin liquid. Other techniques show that molecular motions are also maximized at the bilayer center. However, note that disorder is a static property and implies nothing about motions. For example, one can have a highly disordered structure, such as window glass, which displays little motion (see Chapter 5).

Neutron Diffraction

The specifically deuterated phospholipids can also be studied by neutron diffraction (see 1320). Whereas X-ray scattering reflects the electron distribution, neutrons are scattered by atomic nuclei. The scattering properties of ^1H and ^2H are very different, which permits the localization of deuterated sites in the scattering density profile. For example, the C-5 position can be localized 15 Å from the bilayer center in dipalmitoyl phosphatidylcholine in the gel phase, consistent with an all-*trans* chain configuration (161). By using D_2O as solvent, the location of water in the gel phase has been determined, demonstrating penetration up to the level of the glycerol moiety (1606, 622). The location of ions such as Ca^{2+} has also been studied using neutron scattering (622) (see Chapter 7).

2.3 The Thermodynamics of Lipid Polymorphism

The structural data presented in previous sections show that hydrated lipids exhibit polymorphism. In all the lipid structures, the nonpolar hydrocarbon portions of each molecule are aggregated and the polar headgroups are in contact with water. In this section, we will briefly describe the thermodynamic principles of micelle formation by amphiphilic lipids. The thermodynamic arguments will be summarized diagrammatically in terms of molecular shapes, providing a useful rationale for such diverse questions as why detergents disrupt membranes and how cholesterol affects the phospholipid bilayer.

2.31 The Hydrophobic Force (for review, see 1432)

The major thermodynamic driving force stabilizing hydrated lipid aggregates is the hydrophobic force. Other stabilizing factors are

1. *van der Waals forces*: Short, weak attractive forces between adjacent hydrocarbon chains. The attraction results from interactions between polarizable electrons (induced dipoles).

2. *Hydrogen bonding*: Between polar headgroups of some lipid molecules such as phosphatidylethanolamine (see 115). Intermolecular bridging by divalent cations can also be important in some circumstances with anionic lipids.

These are relatively minor stabilizing factors, however, compared to the hydrophobic force.

The hydrophobic force is the thermodynamic drive for the system to adopt a conformation in which contact between the nonpolar portions of the lipids and water is minimized. This so-called “force” is entropic in origin and results from the unfavorable constraints placed on water as it packs around a nonpolar hydrocarbon.

The structure and dynamics of pure water are complex but are clearly dominated by intermolecular hydrogen bonds (see 1392 for review). When an ion such as Cl^- is placed in water it becomes solvated and water molecules form a hydration shell around the ion. The orientation of these water molecules is unfavorable entropically, but this is more than compensated by a large favorable electrostatic interaction, so the overall free energy change in going from a salt crystal to the dissolved salt is favorable. When a nonpolar substance is dissolved in water, it also causes an unfavorable organization of the water around each molecule. In essence, the water molecules orient themselves to maintain intermolecular hydrogen bonds (each worth about 5-7 kcal/mol), but since those solvent molecules in direct contact with the non-polar solute molecule have fewer water molecules as neighbors, there are substantial configurational constraints on the system. Hence, there is a decrease in the entropy of the system. However, there is no large compensating electrostatic interaction as in the case of ionic or polar solutes. As a result, the net free energy change upon transferring a nonpolar solute from a nonpolar solvent (e.g., heptane) to water is unfavorable due to this entropic effect on the water solvent. Possible models for the way in which water molecules orient about nonpolar solutes are exhibited by crystals of hydrated nonpolar molecules or atoms (e.g., argon) showing water cages or clathrates surrounding the “solute” (see 460).

The unfavorable interaction between nonpolar solutes and water is what is termed the “hydrophobic force,” and thermodynamic measurements can quantify the tendency for nonpolar materials to minimize their contact with water. This is a major stabilizing factor for virtually all biological macromolecular structures, including globular proteins as well as the phospholipid bilayer. The “hydrophobicity” of simple molecules such as alkanes can be quantified simply by measuring the equilibrium distribution of the solute (e.g., ethane) between two solvents, e.g., water and heptane.

Expressing the solute concentration in mole fraction units in water $[\text{X}]_{\text{H}_2\text{O}}$, or hydrocarbon, $[\text{X}]_{\text{HC}}$, we define an equilibrium constant K :

$$\frac{[\text{X}]_{\text{H}_2\text{O}}}{[\text{X}]_{\text{HC}}} = K \quad [2.1]$$

$$\text{and } -RT \ln K = \Delta G_{\text{trans}}^{\circ} = (\mu_{\text{H}_2\text{O}}^{\circ} - \mu_{\text{HC}}^{\circ}) \quad [2.2]$$

The standard state transfer free energy, $\Delta G_{\text{trans}}^{\circ}$, is a measure of hydrophobicity (see 1432). Hydrophobicity has been shown to be proportional to the surface area of contact between water and the nonpolar solute (1212). Larger molecules (e.g., long-chain alkanes) cause a larger perturbation on the water structure because there is more area of contact. Figure 2.16 shows that for a series of alkanes, the hydrophobicity ($\Delta G_{\text{trans}}^{\circ}$) increases in proportion with surface area. Using van der Waals radii to compute the surface area of contact between water molecules and the alkanes, it was computed that $\Delta G_{\text{trans}}^{\circ}$ changes by about $-25 \text{ cal}/\text{\AA}^2$. For straight chain alkanes the hydrophobicity changes by about -800 cal/mole of $-\text{CH}_2-$. In other words, for every increase in chain length of two methylenes, the equilibrium constant changes by about a factor of 10 in favor of the hydrocarbon solvent.

2.32 Micelle Formation

Consider what happens when a long-chain alkane is dissolved in water. Because of the very unfavorable “hydrophobic” interaction described in the previous section, the solubility will be very low. Up to a point, alkanes such as dodecane (C_{12}) can be dissolved in water, but beyond a certain concentration the dodecane will form a separate phase. Further additions of dodecane will simply increase the amount in the separate phase of dodecane and not increase the concentration of dissolved hydrocarbon in the aqueous phase (see Figure 2.17).

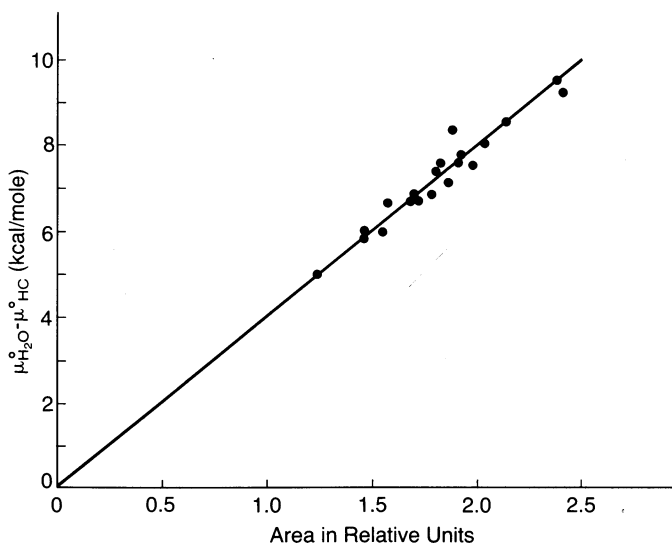


Figure 2.16. Unitary free energy of transfer of hydrocarbons from the pure liquid to aqueous solution at 25°C plotted as a function of relative surface area. The area of isobutane on this scale is 1.45. Adapted from ref. 1212.

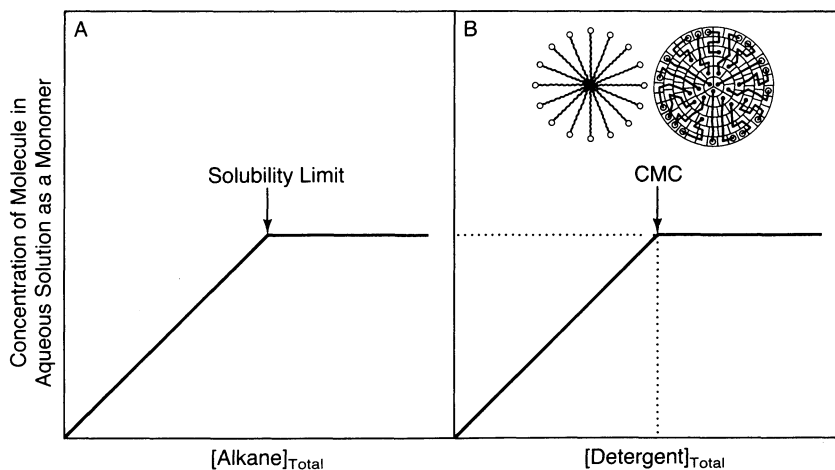


Figure 2.17. Schematic to illustrate the conceptual relationship between a solubility limit and the critical micelle concentration (CMC). Alkanes will dissolve in water up to a defined concentration, at which point a separate phase forms (part A). Amphiphiles, such as detergents, will dissolve up to a point where micelles start to form, defined as the CMC (part B). Also shown in part B are two drawings showing the cross section of a spherical micelle. The drawing on the left is the common way of indicating a micelle, but it is not realistic because the density of material in the center would be much greater than near the edge and the chains cannot pack together in this way. The drawing on the right shows a more realistic distribution of conformations of the hydrocarbon chains in a micelle, based on a statistical theory (ref. 336).

Now consider what happens when an amphiphilic molecule such as dodecyl sulfate (SDS) is added to water. This common detergent has both a nonpolar portion (dodecyl chain) and a highly charged polar group (sulfate) at one end. When the solubility limit for the monomeric form of this detergent is reached, it will also form a separate phase. However, the “phase” in this case is in the form of aggregates, called micelles, dispersed throughout the solution. Because of the highly favorable interactions between the polar headgroup (sulfate in this case) and water, it is preferable to maintain this part of the molecule in contact with water while still excluding water from contact with the nonpolar portion of the molecule. The result is a globular aggregate. The concentration at which 50% of the detergent is in the form of micelles is called the critical micelle concentration, or CMC. Operationally, it is often convenient to define the CMC as the concentration where micelles first appear. The CMC is similar to a solubility limit for the monomeric form of the molecule. Additions of dodecyl sulfate beyond the CMC essentially increase the concentration of micelles.

Lipid aggregates or micelles come in many sizes and shapes. Dodecyl sulfate in water forms spherical micelles with about 60 molecules per micelle. Other detergents or amphiphiles form globular or rodlike aggregates. Phospholipids spontaneously aggregate to form bilayers, and the bilayer is essentially another

micelle form. The reason why biological phospholipids form stable bilayers is not difficult to understand and is described in the next section. First, however, we will quantify the relationship between molecular hydrophobicity and the CMC.

An aqueous solution of an amphiphile might consist of a mixture of species, including monomers ($N = 1$) and various aggregates containing many molecules each. At equilibrium, the chemical potential of the amphiphile in each of the various forms will be the same (see 683, 682).

$$\mu_N = \mu_N^\circ + \frac{kT}{N} \ln (X_N/N) = \text{constant, same for all } N \quad [2.3]$$

where μ_N° is the standard state chemical potential of species with N molecules, X_N is the mole fraction of the amphiphile which is in the aggregated species with N molecules, k is Boltzmann's constant, and T is temperature. For simplicity we will assume a monodisperse system with M molecules per aggregate. That is, there is only one aggregated form which exists ($N = M$) in equilibrium with the monomer. Although this is clearly an approximation, it is not an unreasonable assumption for molecules that form spherical micelles or small bilayer, single-walled vesicles. Now we apply the condition for equilibrium at the critical micelle concentration, and define the CMC as the concentration where $X_1 = X_M$ ($\equiv X_{\text{CMC}}$)

$$\begin{aligned} \mu_1 &= \mu_M \\ \mu_1^\circ + kT \ln (X_{\text{CMC}}) &= \mu_M^\circ + \frac{kT}{M} \ln \left(\frac{X_{\text{CMC}}}{M} \right) \\ \text{or } \Delta G_{\text{mic}}^\circ &= (\mu_M^\circ - \mu_1^\circ) \equiv kT \ln X_{\text{CMC}} \end{aligned} \quad [2.4]$$

A major component of $\Delta G_{\text{mic}}^\circ$ is the hydrophobic transfer free energy due to excluding water from the nonpolar portion of the amphiphile when burying it inside the micelle. Note that a more negative $\Delta G_{\text{mic}}^\circ$ results in a smaller value of X_{CMC} . That is, very hydrophobic molecules tend to aggregate at lower concentrations. In fact, for simple amphiphiles with single alkane chains (e.g., alkyl sulfates such as dodecyl sulfate) the chain length dependence of $\Delta G_{\text{mic}}^\circ$ is very similar to the chain length dependence of $\Delta G_{\text{trans}}^\circ$ for alkyl chains (equation 2.2). Thermodynamically, transferring a nonpolar moiety from water to a liquid hydrocarbon is similar to transferring it to the interior of a micelle. Quantitatively, this means that for each increase in chain length of two methylene groups, there will be decrease in the CMC of about an order of magnitude.

The importance of this to membranes is that for biological phospholipids, which typically have two long alkyl chains per molecule, the hydrophobic component of $\Delta G_{\text{mic}}^\circ$ very strongly favors the aggregated state (the bilayer). The CMC for such lipids is $< 10^{-10}$ M. In other words, for most purposes, the concentration of monomeric phospholipids in equilibrium with the membrane is negligible. Nature has designed special proteins for binding and transferring the monomeric forms of lipids within or between cells, and these are discussed in Chapter 10.

2.33 Micelle Shapes: Why Does a Bilayer Form?

As developed in the previous section, it is clear that biological phospholipids spontaneously aggregate in aqueous solution. We now must consider the problem of what micelle form is most favored. Here it should be noted once again that some biological phospholipids, such as phosphatidylethanolamine with unsaturated fatty acyl chains, do not form stable bilayers when dispersed in water. In order to understand the stability of the bilayer and the possible role of “non-bilayer-forming” membrane components, it is necessary to develop further the thermodynamics of these systems and to discuss the concept of lipid shapes in relation to the constraints of packing lipids together in micelles. Readers interested in a more qualitative, pictorial view can skip directly to the next section.

Why don't membrane phospholipids form globular micelles? In considering the problem of how amphiphilic molecules pack into a particular micelle geometry (e.g., a sphere), it is convenient to consider the packing requirements of the molecule in two parts. First, the nonpolar portion of the molecule has a fixed molecular volume (v) and a maximal length (605). Without any other considerations, this will determine the maximal radius of a spherical micelle, for example, as well as the number of molecules that can fit into the micelle. The second factor that must be considered, however, is the optimal surface area required by the polar headgroup (S_0). For biological phospholipids, the area per molecule in a hypothetical spherical micelle is much larger than the optimal value for headgroup packing and, hence, these amphiphiles do not form stable spherical micelles. In what follows, we will first consider the factors which determine the *optimal surface area per molecule* for an amphiphile at a micelle surface and then see how this value can be incorporated in a *critical packing parameter* which can be used to determine what micelle shape is favored for any amphiphile.

Optimal Surface Area per Molecule

By definition, the distribution of lipids in various aggregated forms (e.g., spherical micelles, bilayers, rods...) is determined by the relative standard state chemical potentials (or mean free energies) of the molecules in each structure, μ_N° . In order to proceed we need to assign a reasonable form for μ_N° , taking the geometry of the micelle into account. Following the development of Israelachvili and his colleagues (683, 682), we will postulate contributions from three terms for the chemical potential of a molecule with average surface area, S , in a micelle with aggregation number N . It is the dependence of μ_N° on the average molecular surface area which allows us to account for micelle geometry.

$$\mu_N^\circ = \underset{1}{\gamma}S + \underset{2}{C/S} + \underset{3}{H_N} \quad [2.5]$$

Terms 1 and 2 are interfacial attractive and repulsive terms, respectively, and term 3 is a bulk energetic term. The origin of these terms is not hard to see. The H (term 3) is the free energy associated with the alkyl chains. To a first approximation, this will be similar in all micelles in which the alkyl chains are buried

in a hydrocarbon-like environment from which water is excluded. It is this term, or more precisely $(H_N - H_1)$, which changes with the chain length of the nonpolar part of the molecule and determines the chain length dependence of the CMC. $(H_N - H_1)$ is the hydrophobicity.

Terms 1 and 2 are energetic contributions due to intermolecular interactions at the water–hydrocarbon interface. These terms vary depending on how close together the lipids are packed in the micelle and, thus, are dependent on micelle shape. The micelle shape which is most favored is that which minimizes the free energy of the system, and it can be, at least qualitatively, understood in terms of the following simple thermodynamic expressions.

Term 1: γS , interfacial surface tension. An attractive term equivalent to the surface tension stabilizing liquid–liquid interfaces in water–hydrocarbon systems. γ is the surface tension expressed as energy per cm^2 and can be estimated as about 50 erg/cm^2 . This is equivalent to the work required to change the interfacial area per cm^2 with a lateral pressure of 50 dyne/cm . Surface tension can also be thought of as a “negative pressure” due to the various attractive molecular interactions at the interface and expressed in units of dyne/cm (50 dyne/cm in this case).

Term 2: C/S , repulsive intermolecular interactions. A crude way of lumping together all sorts of repulsive interactions at the interface, including electrostatic and steric interactions. The essence of the term is that these repulsive interactions vary inversely as the average area per molecule at the micelle hydrocarbon surface. In other words, when the molecules are close together (small area per molecule) these interactions become very large and unfavorable.

These two terms represent what Tanford has called the principle of opposing forces (1432). The drive for the molecules to associate is counteracted by repulsive interactions, lumped into the constant C , which ultimately determines the optimal packing density in the micelle.

The optimal value of S is obtained by setting $(d\mu_N^\circ/dS) = 0$, i.e., minimizing the free energy with respect to molecular surface area. The result gives S , the optimal surface area per molecule:

$$S_o = \sqrt{C/\gamma} \quad [2.6]$$

Even in this oversimplified model, one can see that the molecular constant C determines S_o . For dodecyl sulfate, for example, one would expect a large electrostatic repulsive interaction between the charged sulfates at the micelle surface (e.g., large C) to result in a large value of S_o , especially at low ionic strength. The polar headgroup of this molecule essentially requires a large surface area at the micelle surface to keep the charged groups far apart. This requirement dominates and determines the preferred spherical micelle shape. To see how, consider the following.

Micelle Geometry and the Critical Packing Parameter

Three molecular parameters must be considered in determining the most stable micelle geometry:

1. S_o , the optimal surface area occupied by the molecule at the hydrocarbon interface. This will be, in part, dependent on the solution conditions, especially ionic strength in the case of charged molecules.
2. l , the maximum length of the alkyl chain for simple single-chain amphiphiles and for phospholipids. This will determine the upper limit on micelle size, such as the radius of a spherical micelle or thickness of a bilayer. In no case are micelles considered as having holes or gaps, so the radius of the spherical micelle cannot exceed l , though it could be shorter. Usually this distance is slightly shorter than the fully extended, all *trans*, configuration.
3. v , the molecular volume of the hydrocarbon portion of the amphiphile. The micelle volume enclosed within the envelope of the hydrocarbon–aqueous interface is considered to be equal to Mv , where M is the number of molecules in the micelle.

The surface area available per unit volume is purely a function of micelle geometry and it is this which basically determines what kind of micelle is formed by different amphiphiles. Let us briefly consider some possible micellar forms:

1. *Spherical*: Given the dimensional constraints imposed by the length of the lipid chain, the sphere has the highest area/volume ratio of any form and is favored by lipids with a large value of S_o , such as dodecyl sulfate in water.
2. *Distorted spheres*: Have a lower surface/volume ratio than spheres:
 - (a) *Ellipsoids*: considered to be unlikely (682) because packing would be highly unfavorable in many places (e.g., the edge of oblate ellipsoid)
 - (b) *Globular*: bi-lobed, like two spheres merged. A likely form (682).
3. *Rods and Cylinders*: Even lower surface/volume ratio. The ends would likely be hemispherical so as to exclude water from the nonpolar portions yet maintain reasonable packing.
4. *Bilayer*: Smallest surface/volume ratio, favored by lipids with a large molecular volume, such as lipids with two alkyl chains. Note that disks or flat sheets would be highly disfavored due to contact by water at the disk edge. Spherical bilayer vesicles (liposomes) eliminate this edge and are also favored by being smaller and, hence, entropically preferable to very large bilayer sheets. Some proteins and peptides can stabilize phospholipid disks (see Chapter 3).

The parameter (v/S_o) can be used to predict (or rationalize, in retrospect) which micelle form will be favored for a particular molecule. This is called the *critical packing parameter*, and it is a number containing the packing requirements of the amphiphile, including the volume and length of the nonpolar portion and the optimal surface area of the polar headgroup.

For example, consider a spherical micelle with radius R and containing M molecules.

$$\text{Total micelle area} = MS_o = 4\pi R^2$$

$$\text{Total micelle volume} = Mv = 4/3\pi R^3$$

so the micelle radius

$$R = \frac{3v}{S_0}$$

But the micelle radius must be less than or equal to l ($R \leq l$), the maximum length allowed for the lipid chain, so a criterion for a lipid being able to pack into a spherical micelle is

$$\frac{v}{lS_0} \leq 1/3 \quad [2.7]$$

The same calculation is easily done for cylinder and planar bilayer shapes with the critical values being

$$\text{cylinder: } (v/lS_0) = 1/2 \quad [2.8a]$$

$$\text{bilayer: } (v/lS_0) = 1 \quad [2.8b]$$

This leads to the following predictions, given values for v , l , and S_0 . If the value of (v/lS_0) is less than $1/3$, a spherical micelle is expected; if between $1/3$ and $1/2$, the micelles will be globular or cylindrical; and if the value is between $1/2$ and 1 the lipid should form a stable bilayer. It is the two long acyl chains in biological phospholipids which increase the bulk packing requirement (large molecular volume, v) which results in the stable bilayer. Single-chain phospholipids, like most synthetic detergents, have values of (v/lS_0) between $1/3$ and $1/2$ and do not form stable bilayers. Similarly, diacyl phospholipids with very short chains (e.g., $n = 6$) do not form stable bilayers for the same reason.

Of particular interest is that some biological lipids have values of $(v/lS_0) > 1$ and, hence, do not form stable bilayers. These pure lipids, which have relatively small polar headgroups, form inverted hexagonal phase (H_{II}) aggregates, as we have already seen. The role that such lipids play in biomembranes is uncertain but subject to considerable speculation (e.g., 263, 1041, 533, 303, 267).

2.34 Lipid Shapes

The previous discussion began with a consideration of the thermodynamics of lipid aggregation, but it is clear that an excellent qualitative understanding is possible just by considering the packing requirements of different lipids and the constraints imposed by simple geometric considerations. This is pictorially represented by considering the gross shapes of lipid molecules, in particular, comparing the cross-sectional area of the hydrocarbon portion (crudely, v/l) to the optimal surface area required by the polar headgroup (S_0). The same considerations were used in rationalizing differences in phosphatidylethanolamine and phosphatidylcholine in crystals and in the gel phase bilayer (Sections 2.1, 2.2). Lipids can be simply classed as cones, cylinders, or inverted cones depending on the relative packing requirements of these two regions of the lipid. Figure 2.18 summarizes this modeling with examples. This is essentially a pictorial representation of the thermodynamic consequences as discussed in the previous sections,

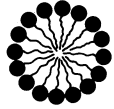

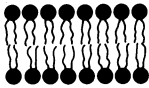

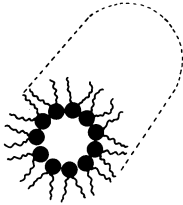

LIPID	PHASE	MOLECULAR SHAPE	CRITICAL PACKING PARAMETER (v/l S.)
Lysophospholipids Detergents	 Micellar	 Inverted Cone	$< \frac{1}{3}$ (Sphere) $\frac{1}{3}$ to $\frac{1}{2}$ (Globular Shapes; Rods)
Phosphatidylcholine Sphingomyelin Phosphatidylserine Phosphatidylinositol Phosphatidylglycerol Phosphatidic Acid Cardiolipin Digalactosyldiglyceride	 Bilayer	 Cylindrical	$\frac{1}{2}$ to 1
Phosphatidylethanolamine (Unsaturated) Cardiolipin - Ca^{2+} Phosphatidic Acid - Ca^{2+} (pH < 6.0) Phosphatidic Acid (pH < 3.0) Phosphatidylserine (pH < 4.0) Monogalactosyldiglyceride	 Hexagonal (H_{II})	 Cone	> 1

Figure 2.18. Polymorphic phases, molecular shapes, and the critical packing parameter for some membrane lipids. Adapted from ref. 263. Drawing kindly provided by Dr. P. Cullis and Dr. M. Hope.

but it allows one to rationalize quickly large amounts of experimental data, at least qualitatively. Quite possibly, these same simple concepts of lipid shape can be used to understand the roles specific lipids play within the bilayer, such as stabilizing regions of high curvature or packing around membrane proteins (263, 1041, 533, 303, 267).

2.4 Lipid Phase Transitions

The thermodynamics of lipid phase transitions has been the subject of numerous experimental (see 829) and theoretical (see 1048, 1163, 1008) studies. Although thermodynamic parameters (e.g., transition temperature, ΔH° , ΔS°) do not give structural information, they must be consistent with any physical chemical model

of the lipid. The ultimate goal of these studies is the quantitative and qualitative description of the biomembrane, including the behavior of the phospholipids in the bilayer and the effects of “perturbants” such as proteins or cholesterol. The goal is far from being realized, but considerable insight derives from concepts and models based on studies of simple systems, starting with aqueous dispersions of pure, homogeneous lipids and proceeding to binary mixtures of different phospholipids as with cholesterol or with specific proteins reconstituted with phospholipids. It must be realized, however, that, generally, biological membranes do not exhibit phase transitions. Hence, much of the material discussed in this section is not directly pertinent to biomembranes. However, the basic physical chemistry of lipids and lipid–protein mixtures is relevant to biomembranes, especially as regards possible lateral inhomogeneities (see Section 4.5) and the way in which components interact in the bilayer.

The most studied lipid phase transition is between the lamellar gel and liquid crystalline phases. However, lipid phase transitions have also been examined between the lamellar and the inverted hexagonal (H_{II}) phases, as well as between other mesomorphic lipid forms. Phase transitions can be induced in several ways, included changes in pressure (215), temperature (e.g., 1444, 1120), ionic strength, or pH (e.g., 194, 1467), depending on the lipid being examined. It is most common to measure the thermally induced or thermotropic transition, not only because it may be biologically meaningful in some cases as in organisms that do not control their own temperature (Section 10.5) but also because the measurements can be made relatively easily and with great precision, and the results directly yield useful information on heat capacities and ΔH° values. These values can be used for comparison with theoretical models. The technique commonly applied is differential scanning calorimetry (DSC).

2.41 Differential Scanning Calorimetry (DSC) (see 56, 949 for reviews)

This technique is of primary importance in obtaining information about the thermodynamics of model membranes and biomembranes. It is used to monitor and characterize changes in physical state in polymorphic lipids and also to characterize the perturbations on pure lipids by the interactions with other materials, such as other lipids, proteins, ions, or small hydrophobic molecules. In DSC, a sample and inert reference are heated independently to maintain an identical temperature in each. The heat for the endothermic gel-to-liquid crystalline bilayer transition, for example, would be required in excess over the heat required to maintain the same temperature in the reference. Differential heat flow is then plotted as a function of temperature. Highly sensitive instruments allow one to use samples of dilute aqueous suspensions of lipids (1 mg/ml, 1 ml sample size). The parameters reported from this technique are:

1. Transition temperature, T_c : the temperature marking the beginning of the transition.
2. Transition midpoint, T_m : where the transition is 50% complete.

3. Transition enthalpy, ΔH : the actual heat required for the entire transition normalized per mole or per unit weight.
4. Heat capacity, C_p : the amount of heat (per gram or per mole) required to raise the temperature of the sample by 1 degree.

Box 2.2 Information Is Obtained from Both the Midpoint and Width of the Transition

For a two-state transition, T_m is defined as the point where $\Delta G^\circ = 0$, so

$$\Delta G^\circ = 0 = \Delta H^\circ - T_m \Delta S^\circ$$

$$T_m = \Delta H / \Delta S^\circ \quad [2.9]$$

which can be used to obtain ΔS° , the transition entropy, from measured values of T_m and ΔH° .

The width of the transition can be an important parameter. This is quantified in terms of the slope of a van't Hoff plot. The data are analyzed in terms of a two-state model (e.g., gel and liquid crystalline phases) and the extent of the transition is used to calculate the fractions in the gel and liquid crystalline state at any temperature. For example, at T_m , the fraction of each state is by definition 0.5. An equilibrium constant, K , is defined as

$$K = \frac{[\text{fraction in liquid crystalline state}]}{[\text{fraction in gel state}]} \quad [2.10]$$

The van't Hoff plot is $\ln K$ vs. $1/T$ and yields as a slope $\Delta H^\circ_{\text{vH}}/R$, giving the transition enthalpy (calories/mol). However, this is normalized per mole of the cooperative unit that is actually undergoing the transition. If lipids were to melt in units of 100 molecules, representing a "cooperative unit," the $\Delta H^\circ_{\text{vH}}$ would be 100 times the "calorimetric" $\Delta H^\circ_{\text{cal}}$, obtained directly by DSC and normalized per mole of lipid molecule.

Thus, a large cooperative unit or highly cooperative thermal phase transition is characterized by a steep slope of the van't Hoff plot (large $|\Delta H^\circ_{\text{vH}}|$) and a very sharp transition. Smaller values of $|\Delta H^\circ_{\text{vH}}|$ result in broader (less cooperative) thermal transitions.

The cooperative unit is defined as follows:

$$\text{Cooperative unit} = \frac{\Delta H^\circ_{\text{vH}}}{\Delta H^\circ_{\text{cal}}} \quad [2.11]$$

A major problem is that a broad transition can result from other causes, most notably the presence of even small amounts of impurities in the lipid bilayer (16). It is quite common, however, to interpret changes in the transition breadth in phospholipid bilayers upon the addition of "perturbants" (e.g., proteins, cholesterol) as being due to changes in intermolecular lipid chain interactions resulting in a decrease in the size of the cooperative unit. Often, the addition of such perturbants will also result in a lower value for $\Delta H^\circ_{\text{cal}}$, and this is usually interpreted as due to a fraction of the lipid being "sequestered" in some manner and not participating in the "bulk" phase transition. Some hydrophobic intrinsic membrane proteins (e.g., Ca^{2+} -ATPase) have this effect, for example (see 526).

Figure 2.19 shows some DSC scans for several phospholipids. For each lipid, the phase transition between the gel and liquid crystalline phases is evidenced by a sharp peak in the heat capacity over a narrow temperature range. The excess heat is required to convert the lipid from the gel to the liquid crystalline phase and the process is usually likened to a simple melting of lipid such as the liquid water/ice transition. The midpoint of the thermal transition is often referred to as a melting temperature. This transition is thought to be a first-order transition which, in theory, is characterized by an infinite heat capacity at the transition temperature. In practice, this is not the case, and the transitions are also often spread over a width of several degrees. It appears that the major cause for the breadth of many of the reported transitions of single-component lipids is the

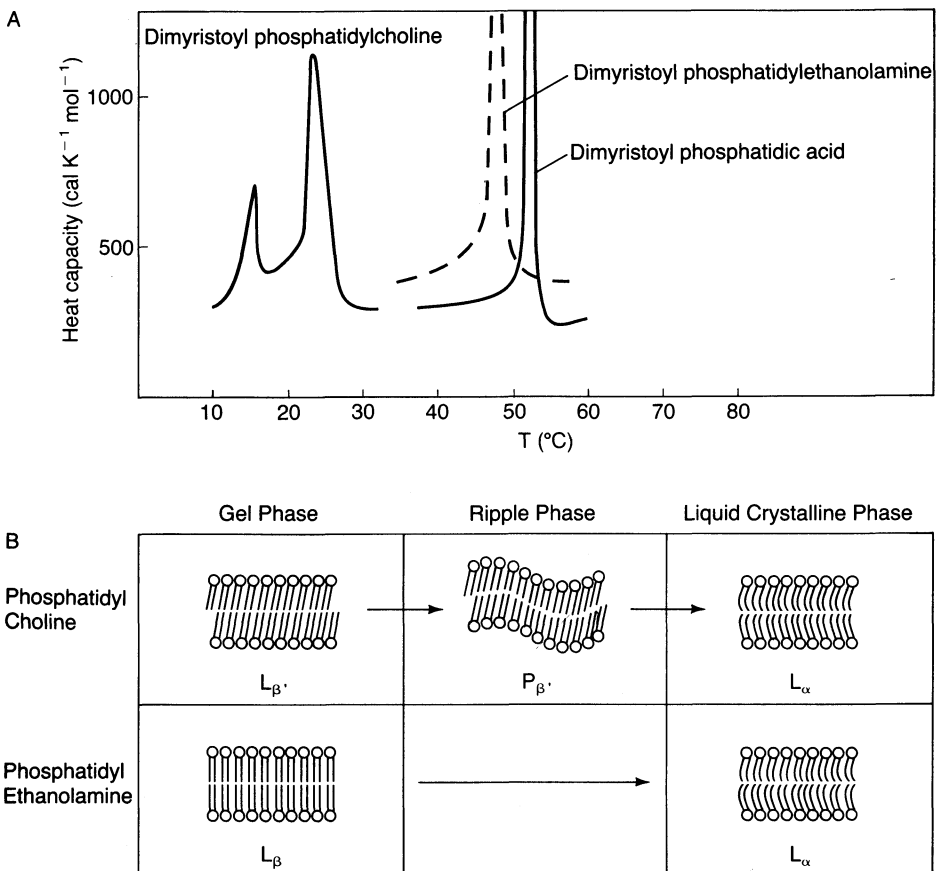


Figure 2.19. (A) Differential scanning calorimetry profiles of three phospholipids. Adapted from ref. 112. Reprinted with permission from "Apparent Molar Heat Capacities of Phospholipids in Aqueous Dispersion," by A. Blume, *Biochemistry* 22, pp. 5437-5438, Copyright 1983, American Chemical Society. (B) Schematic showing the molecular organization of phosphatidylcholine and phosphatidylethanolamine as a function of temperature. Adapted from ref. 112.

presence of minor impurities. For example, the phase transition of highly purified dipalmitoyl phosphatidylcholine is very sharp and is quite sensitive to impurities (16).

The melting temperature of a lipid is determined by a balance of competing factors. Entropically, disordered chains in the liquid crystalline state, characterized by *gauche* conformations, are favored over the highly ordered all-*trans* chain configuration in the gel state. However, attractive chain contacts (van der Waals interactions) are favored by the more ordered gel phase. In addition, the lower cross-sectional area required by the acyl chains in the ordered state results in altering the distance between nearest neighbor polar headgroups. Close interactions may be favorable, as in the case where there are intermolecular hydrogen bonds or bridging divalent metal cations (e.g., Ca^{2+}), or they may be unfavorable, as in the case where bulky groups interact sterically or charged lipids interact electrostatically. In these latter cases, the state of protonation (pH), the ionic strength, and the presence of divalent cations can have dramatic effects on the T_m value for a particular lipid. As the temperature is increased, eventually the entropic effect dominates to stabilize the state with the *gauche* rotamers.

Changes in polar headgroup interactions (e.g., by changing ionic strength) will alter the T_m , since these are primarily changes in ΔH° , and $T_m = \Delta H^\circ / \Delta S^\circ$. For example, high ionic strength will reduce the repulsive electrostatic interactions between the phosphate groups in phosphatidic acid bilayers. However, the effect will be greater (more stabilization) for the gel phase since the polar group density, i.e., charge density, is larger than in the liquid crystalline state. High ionic strength, therefore, results in a larger magnitude for ΔH° , and an increase in T_m . Thus, one can induce phase transitions isothermally under certain conditions by altering the ionic strength or other parameters which affect polar group interactions (see 194).

Table 2.1 and Figure 2.20 summarize some data obtained with pure single component phospholipid systems. Some qualitative remarks are pertinent.

1. The phase transition temperature is most dependent on the fatty acyl chain because of the importance of van der Waals forces in determining the relative stability of the gel and liquid crystalline phases.

Table 2.1. Thermodynamic data for thermotropic phase transitions of a series of diacyl-saturated phosphatidylcholines determined by DSC¹.

Lipid	T_{m_1} (°C)	T_{m_2} (°C)	ΔH_1 (kcal/mol)	ΔH_2 (kcal/mol)	ΔS_2 (cal/°K mol)
DMPC (C_{14})	15.3	24.0	1.3	6.5	21.9
DPPC (C_{16})	35.5	41.5	1.6	8.7	27.7
DSPC (C_{18})	51.0	54.3	1.8	10.4	33.3
DAPC (C_{20})	62.1	64.1	1.7	12.3	37.6

¹From ref. 112. Abbreviations: DMPC, dimyristoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; DSPC, distearoyl phosphatidylcholine; DAPC, diarachidoyl phosphatidylcholine. The subscripts (1, 2) refer to the pretransition (1) and main transition (2).

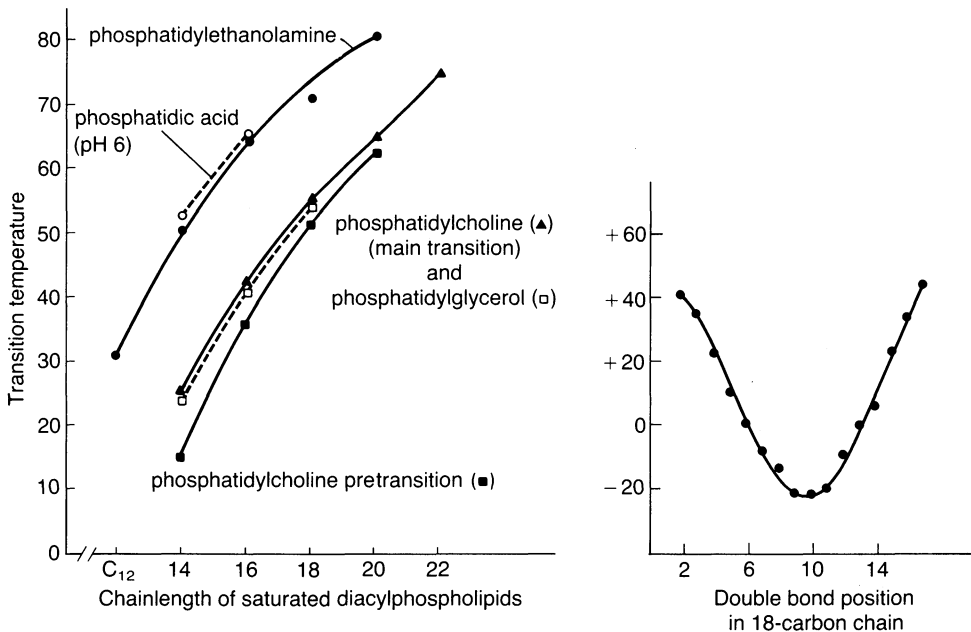


Figure 2.20. Dependence of the phase transition temperature on the chain length of saturated diacyl phospholipids and on the position of the double bond in mono-unsaturated 1,2-dioctadec-*cis*-enoyl phosphatidylcholines. At pH 12, phosphatidic acids have the same transition temperatures as do phosphatidylcholines and phosphatidylglycerols of the same chain length. Data from refs. 112, 73, 698.

- (a) Longer chain lengths result in higher T_m values, because of the increased van der Waals interaction for the longer chains.
 - (b) A *trans* double bond reduces the T_m as this will disrupt the ability of the chains to interact optimally in the gel state.
 - (c) A *cis* double bond has an even larger effect than a *trans* double bond. The magnitude of the effect is dependent on the position of the double bond in the chain, with the maximal effect when the *cis* double bond is in the middle of the chain. Prokaryotic organisms have a wide variety of hydrocarbon chains containing structures such as cyclopropyl or methyl groups which also destabilize the gel state and lower the T_m (1262) (see Chapter 1).
 - (d) Fluorine, often used to replace hydrogen in phospholipids for the purpose of providing an NMR probe, has an influence on T_m similar to a *cis* double bond.
2. Lipids with a large area requirement for the polar headgroup, such as phosphatidylcholines, show a pretransition between two gel states (L_B and P_B) (see 855). These states and the chain tilt exhibited by such lipids are discussed in Section 2.2.

3. Saturated phosphatidylethanolamines melt about 20° higher than do equivalent phosphatidylcholines, presumably due to stabilizing hydrogen bonding possible in the gel state of phosphatidylethanolamine (see 115).
4. The presence of a *cis* double bond in the fatty acyl chains largely removes the difference between T_m values for phosphatidylethanolamine and phosphatidylcholine.
5. Phospholipids with various headgroups (including cardiolipin) have all been shown to exhibit similar phase transitions, as have glycolipids.
6. Charged lipids are very sensitive to ionic strength, pH (if the lipid has a pK in the range being examined), and divalent cations (194). The binding of ions to phospholipids is discussed in Chapter 7.
7. Pressure can also be used to induce a phase transition in phospholipids and alter the T_m . High pressure increases the stability of the gel phase, increasing the T_m of dipalmitoyl phosphatidylcholine for example, by 22°C/kbar (215). The gel phase is stabilized at high pressure because it is more dense than the liquid crystalline phase.
8. At the T_m , the lipid is partially in a gel state and partially in a liquid crystalline state. Experimentally, this has been observed to result in leakiness in vesicles, and the size of the apparent “pores” has been measured in multilamellar liposomes of dimyristoyl phosphatidylcholine to be as high as 18 Å diameter (1489). This is interpreted in terms of packing defects at the transient boundaries between macroscopic gel and liquid crystalline domains of the bilayer. The presence of such “defects” is discussed further in Chapter 9 in relation to membrane fusion.
9. Small vesicles generally exhibit a broad thermotropic transition, presumably due to packing difficulties of the gel state lipids in vesicles with a small radius of curvature.

Finally, there are numerous theoretical approaches to phase changes in lipid bilayers (see 1048, 1163, 1008). Successful models have in common the assumption that chain segments can be described in simple terms as either *trans* or *gauche* (g^+ or g^-). By taking into account hard core short-range repulsive interactions between chains and longer-range attractive interactions, the “melting” of the phospholipid bilayer can be adequately described in terms of the introduction of *gauche* conformers. To some extent these models can be extended to describe the behavior of binary lipid mixtures (1008).

2.42 Lipid Mixtures

The next level of complexity beyond the single lipid is a binary lipid mixture. Many such mixtures have been examined experimentally (see 829). The thermodynamics and structure of such mixtures are relevant to biomembranes since they are informative about the miscibility of different kinds of lipids. A question of particular interest is whether the lipid mixtures found in biomembranes might

spontaneously organize in separate domains of different lipid composition and, hence, with different physical and chemical properties. The concepts of lateral phase separations in biomembranes (see Section 4.54), though far from being established experimentally, derive directly from physical chemical studies of simple lipids and lipid mixtures where such phase separations have been clearly demonstrated (e.g., 783).

Just as the phase transition of homogeneous lipids has been considered analogous to the melting transition of a normal three-dimensional fluid, binary lipid mixtures can be characterized using regular solution theory (829, 830). This has been most successful when applied to mixtures of dissimilar phospholipids.

Phospholipid Mixtures

Thermodynamic studies have clearly demonstrated that dissimilar phospholipids do not mix ideally. In the gel phase, packing requirements may prevent two lipids from being miscible, resulting in clustering or lateral phase separations. Even in the liquid crystalline phase, two lipids may be miscible but the mixture often behaves nonideally. That is, interactions between lipids of the same type are different from interactions between unlike lipids, resulting in preferential arrangements of nearest neighbors.

Nonideal behavior can be characterized by comparing the experimental phase diagram with that predicted theoretically using regular solution theory. The phase diagram is experimentally determined by monitoring the extent of the transition from gel to liquid crystalline phases, often by using differential scanning calorimetry. However, any method which reports the fraction of lipid in each state can be used. Whereas single-component systems show sharp melting profiles, mixtures exhibit considerably broader thermal melts. For mixtures of different composition, the temperatures at which melting initiates and is finished are recorded and used to construct a phase diagram (Figure 2.21A). In between the lines connecting the temperatures where melting is started and where it is terminated is a region where the lipid has partially melted, and liquid crystalline (fluid) and gel (solid) domains are in co-existence. The shapes of these lines depend on the thermodynamic characteristics of the melting of the individual components and of the mixing of the two components. Ideal behavior implies zero enthalpy and entropy of mixing of the two lipids. Equations describing nonideality are relatively easy to derive (see 829, 830), assuming nonideal free energy of mixing, and phase diagrams derived from theory can be compared to experiment. For a mixture of dimyristoyl and dipalmitoyl phosphatidylcholine, the data fit reasonably to curves derived assuming nonideal mixing (nonzero ΔH° for mixing) of these two lipids in the gel phase but ideal mixing in the liquid crystalline phase. These lipids differ by only two methylene groups. More dissimilar lipids behave less ideally. For example, mixtures of dilauroyl and distearoyl phosphatidylcholine (C_{12} and C_{18}) appear to mix nonideally in both gel and liquid crystalline phases. Wilkinson and Nagle (1587) showed that dimyristoyl and distearoyl phosphatidylcholine (C_{14} and C_{18}) are nearly immiscible in the gel

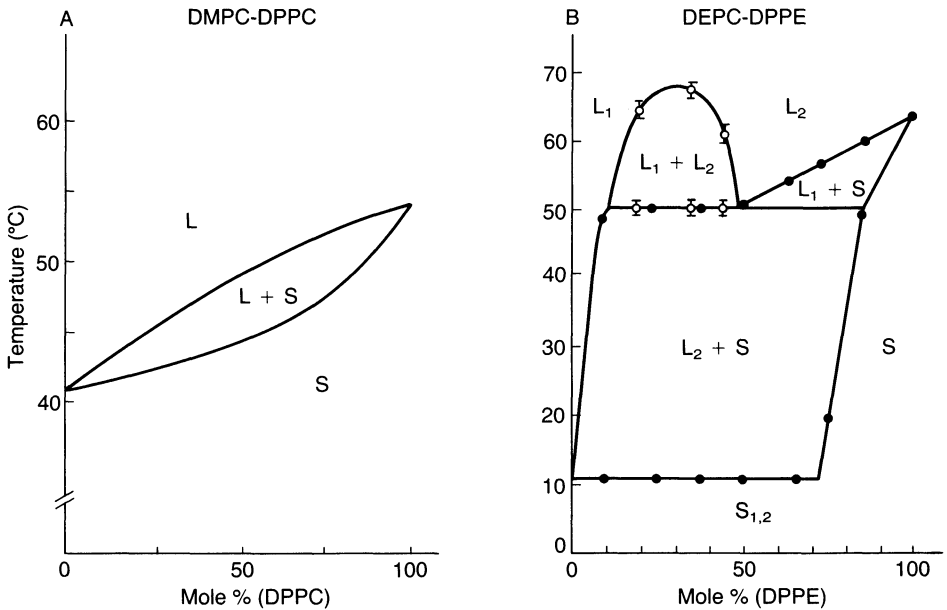


Figure 2.21. Representative phase diagrams of two-component phospholipid mixtures. L, L_1 , and L_2 refer to liquid crystalline phases. S refers to solid-like or gel phase. In part A, the lipids differ only slightly in chain length, and there is complete miscibility in both the gel and liquid crystalline phases. In part B, the lipids are quite different and there is immiscibility in both the gel and liquid crystalline phases. Adapted from ref. 644. Reprinted with permission from "Phase Separations in Phospholipid Membranes," by S. Hong-wei Wu and H. M. McConnell, *Biochem.* 14, pp. 850-851, Copyright 1975, American Chemical Society. Abbreviations: DMPC, dimyristoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; DEPC, dielaidoyl phosphatidylcholine (18:1, *trans*-9); DPPE, dipalmitoyl phosphatidylethanolamine.

phase and that dimyristoyl and dielaidoyl phosphatidylcholine (C_{14} and $C_{18:1,trans}$) are completely immiscible in the gel phase.

Finally, mixtures containing an anionic lipid as one component are dramatically influenced by divalent cations such as Ca^{2+} . The cations apparently form intermolecular cross bridges, resulting in the clustering and lateral phase separation of the anionic lipid component. This can be seen by DSC by the appearance of distinct melting transitions associated with each lipid component (e.g., 1458). This phenomenon can also be directly visualized by fluorescence microscopy (607).

In summary, phospholipids, not surprisingly, behave nonideally in mixtures and show preferences in their nearest neighbors both in the gel and liquid crystalline phases. The significance of these model studies to the distribution of lipids in real biomembranes is not known.

Phospholipids and Cholesterol

The interactions between cholesterol and phospholipids have been extensively studied. Most of the work has used phosphatidylcholines, but other lipids such as phosphatidylethanolamine or sphingomyelin have also been studied (e.g., 398). There is reasonable agreement on the phenomenological description of cholesterol–phospholipid mixtures, but there is no consensus on the interpretation of data in terms of specific structural models. X-ray and neutron scattering show that cholesterol inserts normal to the plane of the bilayer with the -OH group near the ester carbonyl of the lipid (1606) (see Figure 2.22). However, Raman spectroscopy indicates that no actual hydrogen bond is formed with these carbonyls (171). The presence of cholesterol has a substantial effect on the order parameters measured along the lipid hydrocarbon chain by $^2\text{H-NMR}$ (1092) and on the phase transition of the phospholipid (890). FT-IR studies (see 250) show that above T_m , cholesterol decreases the fraction of *gauche* rotamers in the phospholipid hydrocarbon chain, whereas just the opposite is seen below T_m . The ordering phenomenon is due to the difficulty in packing the hydrocarbon chains adjacent to the rigid

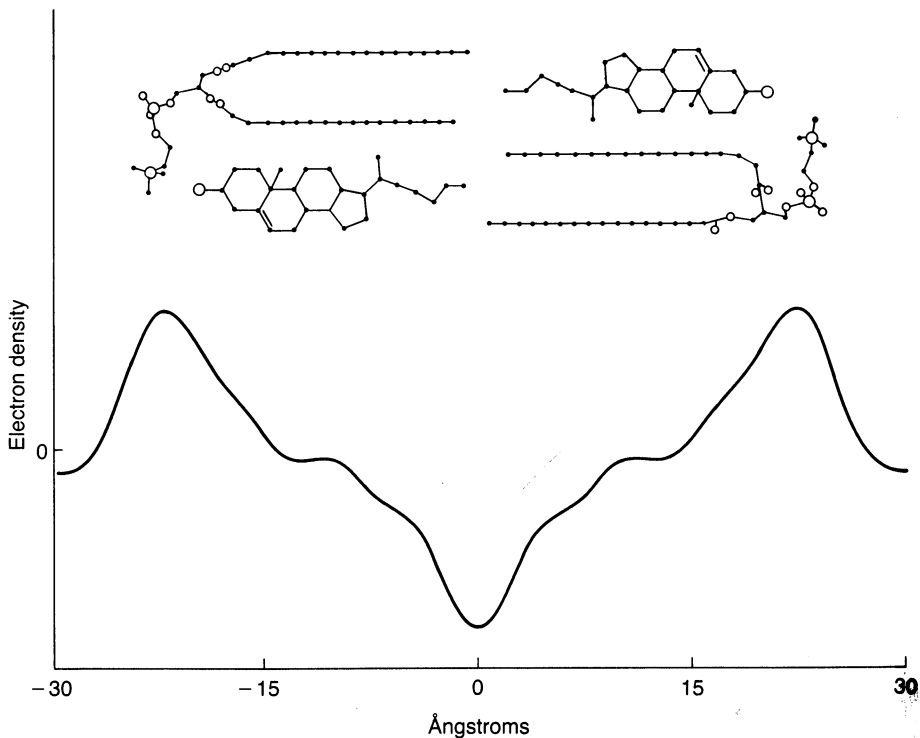


Figure 2.22. Electron density profile of a hydrated bilayer of egg phosphatidylcholine and cholesterol derived from X-ray diffraction analysis. A molecular model consistent with the data is also shown. Adapted from ref. 461.

sterol moiety. In the liquid crystalline state, the sterol results in conformational constraints on the phospholipid chain, whereas in the gel state the sterol inhibits optimal packing of the all-*trans* chain configuration. The result is that lipid-cholesterol mixtures behave in some ways (e.g., disorder) as intermediate between the gel and liquid crystalline states of the pure phospholipid. Basically, cholesterol acts as a “spacer” and reduces the attractive forces between the lipid hydrocarbon chains. In contrast, cholesterol has little effect on the polar headgroups.

A key question of biological relevance is whether cholesterol in biomembranes is distributed randomly in the bilayer or whether it clusters or is concentrated in distinct domains. No simple picture has emerged from the model studies on binary mixtures, but it is clear that phospholipid-cholesterol mixtures do not behave ideally with randomly distributed components. DSC studies on cholesterol mixtures with dipalmitoyl phosphatidylcholine are consistent with the existence of two separate phases below 20 mol% cholesterol and a single phase between 20% and 50% cholesterol (890). Above 50%, no phase transition is observed as the cholesterol completely obliterates the cooperative interactions between the lipid hydrocarbon chains. Various other physical techniques have indicated several phase boundaries in mixtures near 20%, 33%, and 50% cholesterol (839). The structural arrangements within each phase are not known. On the basis of freeze-fracture electron microscopy, Copeland and McConnel (242) have proposed that in the concentration range 0-20% cholesterol with dipalmitoyl phosphatidylcholine, alternating strips containing pure phospholipid and 20% cholesterol exist, resulting in a rippled appearance. It is possible that stable phospholipid-cholesterol molecular complexes may exist but there is no strong evidence to support this. There is evidence, however, that cholesterol interacts preferentially with some phospholipids (1557). Neutron scattering studies (765) indicate that mixtures of cholesterol and dimyristoyl phosphatidylcholine above the phase transition (35°C) are fully miscible, while several distinct phases were observed below the phase transition of the mixture (7°C). Theoretical models (1163) have been moderately successful at duplicating some of the effects of cholesterol on the lipid phase transition parameters (T_m and ΔH°) without including any specific chemical interactions.

In summary, cholesterol-lipid mixtures are complex and polymorphic and there is some evidence for lateral phase separations under certain conditions. How this applies to the role of cholesterol in biomembranes is not clear (see Section 10.4).

Other Mixtures

The influences of numerous hydrophobic compounds on the thermotropic properties of membrane lipids have been studied (see 829). These include numerous drugs, anesthetics, alkanes, alcohols, and fatty acids, as well as natural membrane components such as carotenoids and polyisoprenoids (dolichol, bactoprenol, ubiquinone). Compounds which do not mix well with phospholipids have less effect on the bilayer phase transition, either because they form a separate phase within the bilayer, even in the liquid crystalline phase, or because they do not become significantly incorporated in the bilayer. Cholesterol esters, for example,

are not miscible beyond a few percent with lamellar phase lipids (1453). Physical chemical studies with ubiquinone to locate this molecule within the bilayer have not been conclusive, in part because of uncertainty about whether the quinone is substantially incorporated in the phospholipid bilayer (e.g., 1477, 1391). Whereas many compounds such as polyisoprenoids cause the T_m to decrease because they cannot pack well with gel phase lipids (1477, 1516, 809), other compounds, such as long chain fatty acids and alcohols cause the T_m to increase (889). Some small hydrophobic molecules such as short-chain alkanes appear to form a separate phase in the middle of the bilayer and result in a decrease of the T_m (950).

2.5 Model Membrane Systems

Numerous model membrane systems have been developed for studying the properties of pure lipids, lipid mixtures, and reconstituted lipid-protein mixtures. These model systems can be grouped as (1) monolayers, (2) planar bilayers, and (3) liposomes or vesicles. Each of these systems and their many variants have advantages and disadvantages, and information from each kind of system has been valuable in our developing concepts of the biomembrane. In this section, some of the model systems and their uses are briefly summarized.

2.51 Monolayers at an Air-Water Interface

Many kinds of molecules with substantial non-polar character will be adsorbed at the air-water interface (see 53). This can take the form of a layer only one molecule thick and can be studied as such at the air-water interface or the monolayer can be transported to other supports. Phospholipids and other amphiphilic molecules form an oriented monolayer with the polar portions in contact with the aqueous phase and the hydrocarbon chains extended above. Phospholipids form an insoluble monolayer, since the concentration of the lipid in the aqueous subphase is essentially negligible. This is studied traditionally in a Langmuir film balance or trough, which has a movable float on one side used to control the surface area available to the monolayer (see Figure 2.23). One can use a known amount of lipid to form the insoluble monolayer by, for example, dropping an aliquot of lipid dissolved in volatile solvent on the aqueous surface. The Langmuir film balance allows one to accurately measure the surface area (A) and lateral pressure (π) required to maintain the monolayer. The major advantage of monolayers is the ability to vary the surface density and pressure, and the system has been especially useful in studying the physical chemistry of "surface-active" lipids as well as the enzymology of soluble enzymes such as lipases (324) which act at the lipid-water interface. Although proteins can be incorporated into monolayers, the system is less suited for most studies of intrinsic membrane proteins. Monolayers are usually characterized by "pressure-area" curves. Examples are shown in Figure 2.24. These experimental plots show two important characteristics:

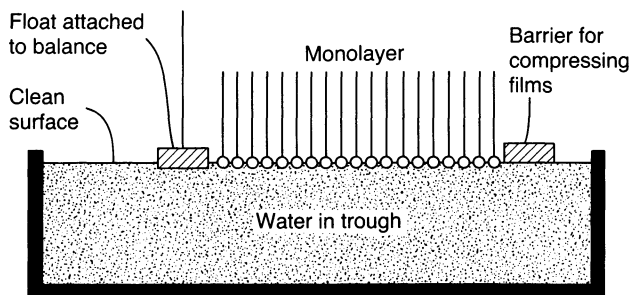


Figure 2.23. Schematic of a monolayer in a Langmuir trough. The polar headgroups are depicted as being in the aqueous phase and the nonpolar chains sticking up. Adapted from ref. 482. "Insoluble Monolayers at Liquid-Gas Interfaces," by G. L. Gaines, Jr., Copyright © 1966 by Interscience Publishers. Reprinted by permission of John Wiley & Sons, Inc.

1. *Discontinuities*, which are due to apparent phase transitions from expanded "liquid-like" or "gas-like" phases at low monolayer densities to more "solid-like" compressed forms at high monolayer densities. The physical properties of monolayers are related to those of bilayers (896, 1160, 505).
2. *Collapse point*, at which the molecules are packed to their maximum density and any further compression results in breakdown of the monolayer. This gives the minimum surface area per molecule.

Saturated alkyl chains occupy approximately 20 \AA^2 , as seen also in the X-ray structural analyses. *Cis*-unsaturated chains have a larger minimum surface area due to chain packing. The minimum surface area required by a disaturated phospholipid would therefore be expected to be $40\text{--}45 \text{ \AA}^2$, which would correspond to a gel phase in the bilayer. The minimum area required by a lipid with *cis* unsaturation would be closer to 60 \AA^2 . Large polar headgroups, such as in phosphatidylcholines or in gangliosides, result in increased minimal surface area requirements.

The phase transition from the fluid "liquid-expanded" phase to the "liquid-compressed" phase has been subject to considerable theoretical and experimental investigation (see 1160, 53). At a given pressure, monolayers can be induced to undergo a thermotropic phase transition which can be directly compared to that observed in the bilayer. For example, at 15 dynes/cm, a monolayer of dipalmitoyl phosphatidylcholine will undergo a phase transition with $T_m = 27^\circ\text{C}$ and $\Delta H^\circ = 8.7 \text{ kcal/mol}$. This compares with the corresponding bilayer transition of $T_m = 41^\circ\text{C}$ and $\Delta H^\circ = 8.7 \text{ kcal/mol}$. Pink (1160, 505) has shown that a relatively simple model, which takes into account a very small attractive interaction between the two monolayers comprising the bilayer, can account for the difference in T_m between the monolayer and bilayer. This is an important point and suggests that from a thermodynamic viewpoint, the bilayer behaves largely as two nearly independent monolayers. Hence, it is conceivable that if the two monolayer leaflets making up a biomembrane were of very different composition, the physical properties could also be different and, to a limited extent, uncoupled. Of course,

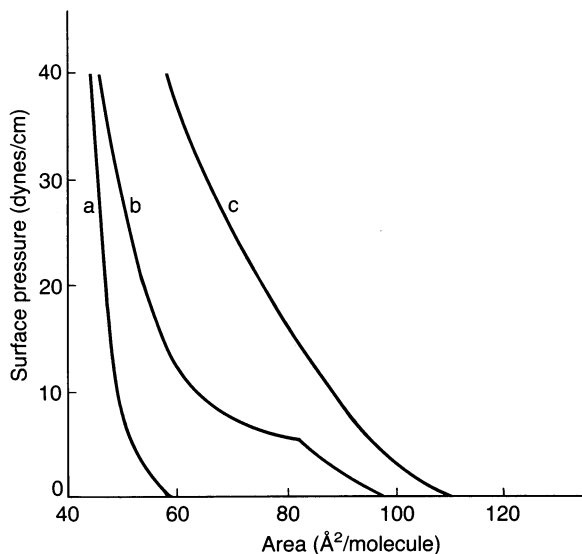


Figure 2.24. Representative pressure–area curves obtained with phospholipid monolayers: (a) Dibehenoyl phosphatidylcholine (22:0), which remains in a solid-like phase; (b) dipalmitoyl phosphatidylcholine (16:0), which exhibits a phase transition from liquid-like to solid-like upon compression; (c) egg phosphatidylcholine, which remains in a liquid-like phase. Adapted from ref. 698. “Introduction to Biological Membranes,” by M. K. Jain and R. C. Wagner, Copyright © 1980. Reprinted by permission of John Wiley & Sons, Inc.

transmembrane proteins or other components could modify this. Membrane asymmetry is discussed in more detail in Chapter 4. Finally, the model is consistent with the internal pressure of the bilayer as being 2×15 dynes/cm = 30 dynes/cm. The thermodynamic forces stabilizing the bilayer result in an internal pressure due to the steric repulsions of both the hydrocarbon chains and polar headgroups. This has been estimated (see 1160) between 12.5 and 50 dynes/cm, balanced formally by the attractive surface tension (683).

It should be noted that the pressure, π , measured for a monolayer is actually the difference in surface tension between the surface with the monolayer (γ) and the pure air–water interface (γ_0):

$$\pi = (\gamma_0 - \gamma)$$

The spontaneous formation of a monolayer at the air–water interface invariably results in a reduction of the surface tension. Surface tension can be viewed as a negative pressure due to the attractive interactions of the molecules at the interface, and this is lowered by the “surfactant” making up the monolayer. Dipalmitoyl phosphatidylcholine, for example, is a major component of lung surfactant, which reduces the work required to change the surface area of the lung during breathing to near zero ($\gamma = 0$) (598).

Most exciting visually are the data from a technique called epifluorescence in which the fluorescence of a lipid probe doped into a monolayer is monitored by fluorescence microscopy (946, 1145). Probes are used which partition favorably into either the gel or liquid crystalline phase. When both phases coexist, this is visualized by light and dark areas due to differences in the fluorescence intensity from the probe in the different regions. Very striking periodic arrangements of solid-phase regions begin to appear in monolayers of dipalmitoyl phosphatidylcholine at pressures as low as 5 dynes/cm (20°C), and as the pressure is increased, these regions grow at the expense of the fluid-like regions. Figure 2.25 shows an example where the monolayer has an average molecular area of about 60 Å². Studies on these monolayers not only show the coexistence of phases but also demonstrate long-range order, possibly stabilized by electrostatic interactions (946).

2.52 Monolayers on a Solid Support

Monolayers formed at the air–water interface can be transferred to a solid support such as an alkylated glass coverslip simply by bringing the glass into contact with the monolayer (946). The alkylation of the glass provides a nonpolar surface. The lipid polar headgroups remain in contact with water. These monolayers can be transferred to the solid support at different surface pressures (π) and then exam-

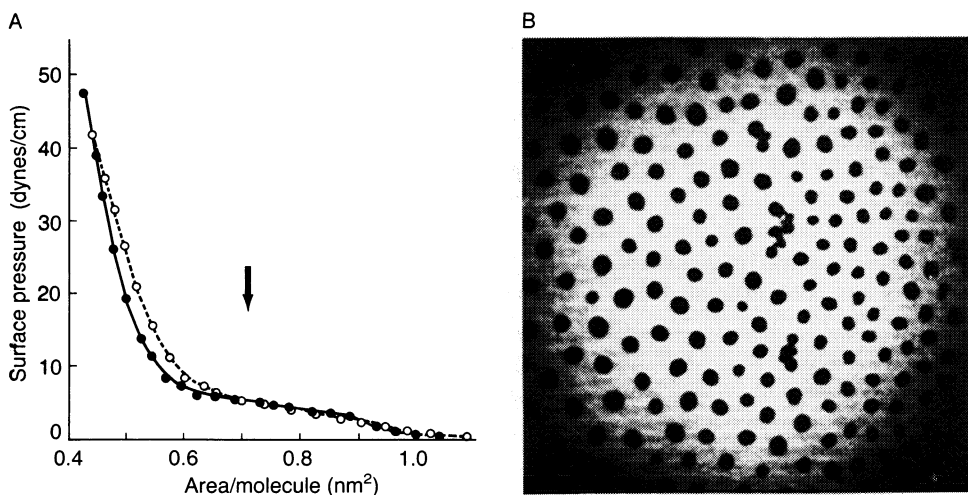


Figure 2.25. (A) Pressure–area curve for dipalmitoyl phosphatidylcholine spread on distilled water at 20°C. The sample contained 2% mol of a fluorescent phospholipid, NBD-egg phosphatidylethanolamine (see Table 5.1). At the point of compression indicated by the arrow in part A, the epifluorescence of the monolayer was photographed (part B). Dark regions are solid-like, and the strongly fluorescent regions are liquid-like domains in which fluorescent probe is preferentially dissolved. The regular pattern is thought to be stabilized by electrostatic forces. The field of view is about 200 μm across. From ref. 946. Photograph kindly provided by Dr. H. McConnell.

ined. Several studies (946, 1145, 1565, 1450) have demonstrated that the dynamic and thermodynamic properties of these supported monolayers as well as monolayers at the air–water interface are similar to those observed for bilayers. Fluorescence methods have also been developed to examine the orientation of molecules in the supported monolayer (1450).

2.53 Planar Bilayer Membranes

Traditionally, planar membranes are produced by painting a concentrated solution of phospholipid in a solvent such as decane over a small hole (~1 mm diameter) in a nonpolar partition (e.g., polystyrene) separating two chambers containing aqueous buffers. Much of the excess solvent disperses in the medium and, under appropriate conditions, the lipids spontaneously form a bilayer across the small hole. These membranes are frequently called bimolecular lipid membranes or BLMs. Because of the lack of light reflectance, they are also called black lipid membranes. Planar membranes formed in this way can be used to study membrane proteins (e.g., ion channels, 987, 988), but they have the disadvantage of containing an unknown amount of residual solvent and they are also unstable, particularly in the presence of small amounts of detergents or other impurities. An alternate procedure for making planar membranes has been developed to avoid the difficulties with the solvent and also to facilitate the incorporation of intrinsic membrane proteins (1009, 1010, 1011, 246). The planar membrane is made across a small orifice from a monolayer at an air–water interface by a simple dipping procedure using either a small pipet (Figure 2.26) or a nonpolar partition with a small hole. Monolayers containing purified membrane proteins can be formed from vesicular protein–phospholipid dispersions and these monolayers used to form the planar membrane. In addition, membrane vesicles containing ion channels can be fused with a preformed planar membrane, incorporating the protein to be studied into the model membrane (see Section 8.14).

The overwhelming advantage of using planar membranes is for making electrical measurements, and this system is particularly valuable for studying pores, channels, or carriers that facilitate or catalyze the transfer of charge across the bilayer from one compartment to the opposing compartment (see Chapter 8). Electrodes are easily utilized in the aqueous chambers, solutions easily changed, and the measurement of current and/or voltage is very accurate and sensitive. One variation of the use of planar membranes is to form them over the tip of a small pipet (diameter < 1 μm) (Figure 2.26). This small patch of bilayer in the “patch pipet” can be used for very sensitive electrical measurements with reconstituted purified membrane proteins, such as the acetylcholine receptor, yielding data on the properties of individual molecular events (1402) (see Section 8.14).

2.54 Planar Bilayer Membranes on Solid Support

Phospholipid bilayers can also be formed on solid hydrophilic supports such as oxidized silicon wafers by the sequential transfer of two monolayers from an air–water interface (1430). The bilayer probably has a water layer between the

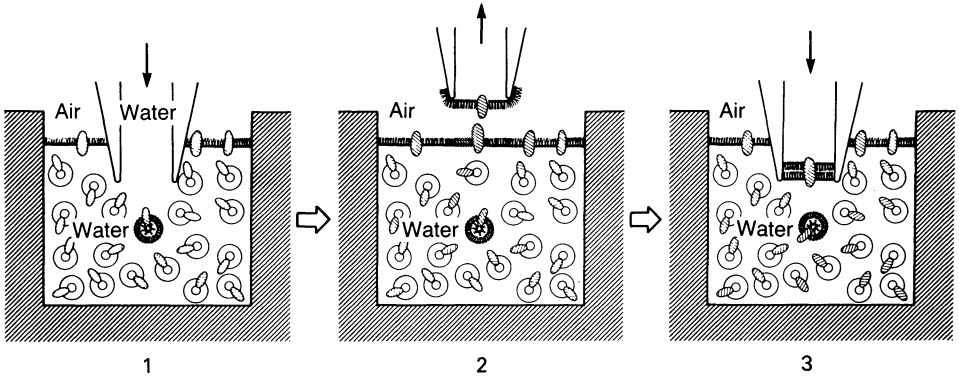


Figure 2.26. Schematic showing the process of forming a bilayer at the tip of a patch pipet. A monolayer is derived from reconstituted phospholipid vesicles containing a protein of interest (see Chapter 3). The pipet is dipped into the solution under positive pressure (Step 1). Upon withdrawing the pipet (Step 2), the monolayer adheres to the pipet by interactions with the lipid headgroup, leaving the hydrocarbon tails in contact with air. The pipet is then re-immersed into the solution (Step 3), resulting in the formation of a bilayer. From ref. 1402. Reprinted with permission from "Single-Channel Recordings from Purified Acetylcholine Receptors Reconstituted in Bilayers Formed at the Tip of Patch Pipets," by B. A. Suarez-Isala, et al., *Biochemistry* 22, p. 2320, Copyright 1983, American Chemical Society. Figure kindly provided by Dr. M. Montal.

solid support and the lipid polar headgroups. By starting with a monolayer with a reasonable average molecular area, a stable bilayer can be made in this fashion. These bilayers are convenient for physical chemical studies of pure lipids and, possibly, eventually for studying membrane proteins. Bilayers of dipalmitoyl phosphatidylcholine made in this way exhibit sharp thermal phase transitions corresponding to the main and pre-transitions observed in lamellar lipid dispersions. These bilayers are especially well suited for measuring the lateral diffusion of membrane-bound molecules using fluorescence techniques (see Chapter 5), and the results are consistent with data from other systems.

2.55 Liposomes

The term "liposome" can be defined as any lipid bilayer structure which encloses a volume (for reviews see 312, 1417, 649). Many phospholipids when dispersed in water spontaneously form a heterogeneous mixture of vesicular structures which contain multiple bilayers forming a series of concentric shells. These were the first "liposomes" to be characterized and are now termed multilamellar vesicles or MLV. Of greater utility are single-walled or unilamellar vesicles, which can be prepared by a variety of methods. These can be generally characterized as small unilamellar vesicles (SUV), with diameters in the range 200 Å to 500 Å, and large unilamellar vesicles (LUV) with diameters from 500 Å to 5000 Å. Very large or cell-sized phospholipid vesicles can also be prepared and used as model membranes, with diameters as large as 300 μm (1031).

The primary uses of liposomes are (1) as model membranes in which proteins are incorporated and studied and (2) to encapsulate solutes for such uses as drug delivery systems. Liposomes are characterized by their lipid composition, their average diameter, and the extent of size heterogeneity in the population. Sizing is performed by (1) gel filtration chromatography, (2) light scattering, (3) ultracentrifugation, or (4) electron microscopy (see 1306, 1214). Of particular interest to those interested in the ability of liposomes to encapsulate solutes are the (1) *captured volume*, or volume of entrapped solute per mole of lipid, and (2) *encapsulation efficiency*, or the percentage of the aqueous volume inside the vesicles. The former parameter increases with liposome diameter, and the latter is obviously proportional to lipid concentration.

Some of the characteristics and preparative techniques for liposomes are summarized below (see 312, 1417, 649).

(1) *Small unilamellar vesicles (SUV)*: These are usually prepared by sonication of aqueous dispersions of phospholipid (659). The vesicles can be further sized by gel filtration chromatography or by glycerol gradient centrifugation (529) to yield a highly homogeneous distribution with diameter ~ 250 Å. Alternatively, these can be prepared by quickly injecting an ethanolic solution of lipids into the aqueous phase. A French press can also be used to prepare SUVs.

Although the population homogeneity is advantageous, the small size can be a disadvantage or, at the least, a factor to be considered. The small radius of curvature of SUVs results in packing difficulties of lipids. The surface area of the outer monolayer of lipids is almost twice that of the inner monolayer, and about 70% of the lipids on SUVs are in the outer leaflet. Lipids with an "inverted cone" shape (see Figure 2.18), such as phosphatidylcholine or sphingomyelin, will preferably partition into the outer leaflet, resulting in lipid asymmetry. This packing phenomenon is also likely responsible for the asymmetric orientation of some membrane proteins reconstituted in vesicles (both SUVs and LUVs). The thermotropic phase transition of SUVs is broadened (841), presumably as a result of the effect of the small radius of curvature on the transition cooperativity. Typical capture volumes are quite small for SUVs, in the range of 0.5 to 1 liter/mol (312). Some properties of SUVs are summarized in Table 2.2 and Figure 2.27.

(2) *Large unilamellar vesicles (LUV)*: The need for larger vesicles became obvious in the 1970s and since then numerous procedures have been devised to produce LUVs. The method of choice may depend on the lipid composition but certainly depends on the intended use for the liposomes. For example, a procedure which results in a dilute liposome solution is not suitable for drug encapsulation. On the other hand, techniques involving organic solvents are not suited for protein reconstitution. Techniques employed include the following (see 312, 1417, 649).

(a) *Detergent dialysis or dilution* (390, 993, 1475, 25): By far the most popular method utilized for biochemical studies because it is relatively easy and is compatible with the inclusion of proteins for reconstitution in the resulting LUVs. Excess detergent is codispersed with the lipid (or lipid plus protein) and the detergent is then removed. Detergents with a high CMC, i.e., in equilibrium with a high concentration of monomer (mM range), can be removed almost

Table 2.2. Parameters of SUVs prepared from egg phosphatidylcholine¹.

Parameter	Value
Molecular weight (hydrodynamic)	1.88×10^6
Partial specific volume	0.9848 ml/g
Outer radius	99 Å
Inner radius	62 Å
Outer monolayer thickness	21 Å
Inner monolayer thickness	16 Å
Number of lipid molecules	
outer monolayer	1,658
inner monolayer	790
Surface area per lipid headgroup	
outer monolayer	74 Å ²
inner monolayer	61 Å ²
Acyl chain cross section at bilayer center	
outer monolayer	46 Å ²
inner monolayer	97 Å ²
Anhydrous bilayer thickness	37 Å

¹Data from ref. 660.

entirely by dialysis (e.g., cholate, deoxycholate, octylglucoside). Detergents with a low CMC (e.g., Triton X-100) can be removed in part by using a hydrophobic resin such as BioBeads, which specifically sequesters the detergent (e.g., 1475). In some cases, simple dilution of the mixture will suffice to reduce the final detergent concentration to a point where the lipids spontaneously vesiculate. The liposome size depends not only on the lipid and detergent utilized, but also on the kinetics of detergent removal (1475). Typically, cholate gives smaller LUVs, 500-800 Å diameter, and octylglucoside gives somewhat a larger population, 1,000-2,000 Å. Residual impurities can be a problem (1120). Capture volumes are much larger for LUVs than SUVs, being in the range 5 to 20 liter/mol (312).

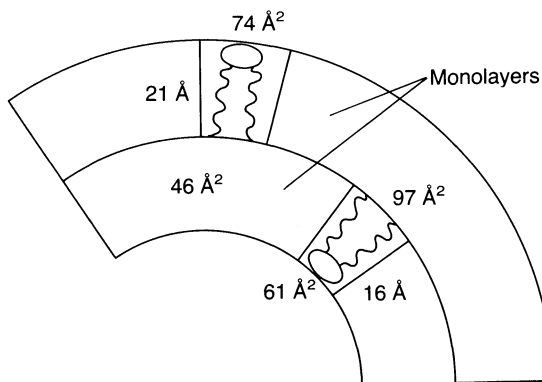


Figure 2.27. Cross section of the bilayer in a small unilamellar vesicle, showing various dimensions. Adapted from ref. 660.

(b) *Infusion and reverse phase evaporation* are separate techniques which involve the use of nonpolar solvents to form LUVs (see 312, 1417). These techniques are not well suited for making model biomembranes with proteins.

(c) *Fusion methods* include several techniques in which SUVs are caused to fuse to form larger liposomes. These techniques include freeze/thaw cycling (898), which is generally applicable and can be used with some proteins, and the use of Ca^{2+} to cause fusion of SUVs made of phosphatidylserine (1119).

(d) *Addition of short-chain phosphatidylcholines* (479, 478) to about 20% of the total lipid has been shown to convert multilamellar bilayers to stable LUVs.

(e) *Addition of fatty acids or detergents* under some circumstances will cause SUVs to fuse to form LUVs (724, 1310). The addition of fatty acids can also result in the incorporation of proteins in the LUV bilayer (1310).

(f) *Rapid extrusion* of multilamellar dispersions through polycarbonate filters can also yield LUVs with diameters in the range 600-1000 Å (648, 649). This method has many advantages.

(g) *Transient increase in pH* causes vesiculation of phosphatidic acid and causes mixtures containing phosphatidic acid to form both SUVs and LUVs (606).

(3) *Cell-size unilamellar vesicles* are formed by simple hydration of lipids or lipid-protein mixtures at low ionic strength (1031). Sizes range from 0.1 to 300 μm, with a capture volume up to 300 liter/mol. They are reported as sufficiently stable to be sorted by centrifugation according to size and can be impaled by microelectrodes for electrical measurements.

(4) *Multilamellar vesicles* are generally not used because of the advantages of single-walled vesicles. Multilamellar vesicles are osmotically active (1621), but the complexity of the many internal volumes makes interpretation difficult.

2.6 Chapter Summary

It is essential to understand the physical chemical behavior of the lipid bilayer in order to appreciate many of the properties of biological membranes. To a large extent, the properties of individual lipids when studied in isolation or in lipid mixtures can be understood qualitatively in terms of shape theory. This is a pictorial way of summarizing information about the way in which the lipid molecules interact. Such considerations are likely to be important even in highly complex biological membranes, and for this reason the study of pure lipids and simple mixtures is relevant to biological systems. Lipids are polymorphic and can exist in structurally distinct phases depending on temperature, pressure, ionic strength, and composition. The lipid bilayer in biological membranes most closely resembles the lamellar liquid crystalline phase which has been characterized for simple lipids and lipid mixtures.

The lipid bilayer is stabilized by the hydrophobic force, which favors structures which minimize the surface area of contact between water and the nonpolar acyl chains. Intermolecular interactions within the polar headgroup region can also be important, including hydration and hydrogen bonding between lipids. A number

of spectroscopic techniques are useful for characterizing the structure and dynamics of the bilayer, including NMR and Raman spectroscopies.

There are several model membrane systems which have been experimentally useful. These include monolayers, planar bilayers, and liposomes. Each has advantages and disadvantages, and each has been useful in extending our understanding of biological membranes.