Polymerization of Preformed Self-Organized Assemblies

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Introduction

The self-assembly of amphiphiles in water creates various lamellar and nonlamellar assemblies depending on concentration, temperature, and pressure.[1−3] Although the concept of self-assembly is certainly not a new idea in the life sciences, it can also be usefully employed for the synthesis of new materials. The organized nature of hydrated amphiphiles offers several attractive features for applications in both biological and materials sciences, e.g., catalysis, separations, surface modification, therapeutics, and diagnosis, as well as model systems for probing signal transduction and molecular recognition, among others. Advances in each of these areas is a consequence of fundamental and applied research in many laboratories. Indeed in recent years a critical level of activity has been attained that appears to permit even more rapid advances in the future. In many cases the potential utility requires a means to make the self-assembled systems more robust. Sometimes referred to as membrane stabilization, it is frequently more useful to explicitly consider the objective in terms of either colloidal and/or chemical stability. In some cases the desired properties can be attained through surface charge or the association of polymers at the assembly surface, whereas in other instances polymerization of the assembly itself is more appropriate. This Account will focus on the latter case with particular emphasis on a strategy that relies on the formation of a self-organized assembly from designed reactive amphiphiles and the subsequent polymerization of the amphiphiles in the assembly.

Hydrated amphiphiles can yield quite complex lyotropic liquid crystals including lamellar (bilayer) and inverted nonlamellar phases, i.e., the inverted hexagonal (H_{II}) and various bicontinuous cubic (Q_{II}) phases. Such morphologies have also been observed for block copolymers, macromolecules composed of blocks of one homopolymer associated with a second homopolymer, that are characterized in part by a general tendency of each block to self-aggregate and form domains.[4,5]

The transition between the lamellar and inverted nonlamellar phases of hydrated lipids requires a change in the geometry of the lipid interface. These thermotropic transitions usually occur between the disordered lamellar (L_α) phase and a disordered inverted nonlamellar phase. Since the transition is between two disordered phases, the transition enthalpies and entropies are small. As a consequence such transitions are very sensitive to molecular perturbations. Lateral stress imbalance can arise under conditions where either the effective headgroup size is reduced or the volume occupied by the acyl chains is increased. The transition between phases can be detected by a number of experimental techniques including differential scanning calorimetry (DSC), ^{31}P NMR, ^{2}H NMR, X-ray diffraction, freeze-fracture electron microscopy, and cryotransmission electron microscopy.

It is proposed that the phase of a lipid assembly is determined by two opposing forces: curvature energy and geometry-dependent energy terms.[6,7] The geometry-dependent energy terms include hydrocarbon packing energy, van der Waals attraction energy, electrostatic repulsion energy, and hydration repulsion energy. Since the opposed forces cannot be simultaneously minimized, lipid systems are said to exist in a state of frustration. As the condition of the system changes, the sum of the forces changes until they can be reduced by a change of the morphology, i.e., a phase transition. One may wonder if the balance of forces in such systems could be maintained

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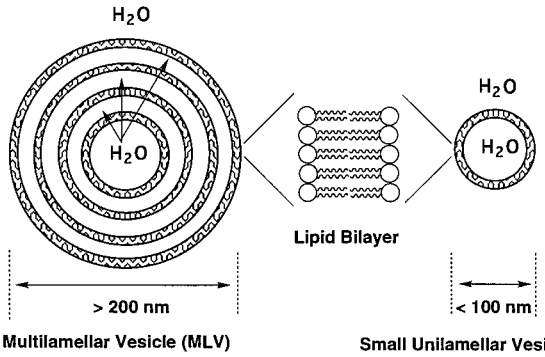
while trying to "stabilize" the assembly via polymerization. Fortunately, the effective methods for such polymerizations were first demonstrated in the more forgiving lamellar phase and the related bilayer vesicles. The advent of accessible synthetic lipids, and the reports of the successful polymerization of fatty acid monolayers, made it clear by the late 1970s that polymerization of lyotropic liquid crystals could be attained at least in lipid bilayers (see reviews).[8−10] These studies provided the background for the successful polymerization of nonlamellar phases in the 1990s.[11−13]

Polymerization of Hydrated Bilayers

The lamellar phase consists of extended bilayers of solvated amphiphiles with periodic smectic-like order. However, for experimental convenience most polymerization studies have focused on lipid bilayer vesicles, i.e., liposomes. A lipid vesicle is a nearly spherical lipid bilayer shell that encloses an aqueous volume. The bilayer shell can be composed of tens of thousands of lipids with their hydrophilic headgroups exposed to water and their hydrophobic tails aggregated in a manner to reduce exposure to water (Scheme 1). Lipid vesicles with diameters of 30 nm up to several hundred nm can be prepared by detergent dialysis of lipid/detergent mixed micelles, ultrasonication, or extrusion of extended bilayers. Although the lipids employed to form vesicles are generally double chain amphiphiles, ion pairs of oppositely charged single chain amphiphiles can also be effective.[14] The low water solubility of the lipids constrains them to the bilayer, where they laterally move past one another, rather like a milling crowd. The lateral diffusion rate depends on the lamellar phase.[15] The lipid diffusion coefficient, D, is ca. 10^{-2} μm^2 s^{-1}, when the sample temperature is below the main phase transition temperature, T_m, i.e., the transition from the solid-analogous phase (L_β) to the liquid-crystalline phase (L_α), whereas at temperatures greater than the T_m the D is ca. 1 μm^2 s^{-1}. The lipid bilayer in the fast diffusion regime provides an organized structure for polymerization reactions which is sufficiently dynamic to permit monomers to diffuse to the growing polymer chain end. Electron microscopy and light scattering were used to demonstrate that polymerization does not significantly alter the shape or diameter of vesicles. However, polymerization of vesicles can dramatically alter their properties.

Polymerizable groups have been incorporated into bilayer-forming amphiphiles by chemical synthesis (see reviews).[8−10] Subsequent formation of bilayer assemblies yields a two-dimensional array of the polymerizable groups. In principle and practice the polymerizable moiety can be positioned anywhere along the lipid tails or linked (covalently or electrostatically) to the headgroup. Polymerization of the lipid tails usually leads to abolition of the bilayer T_m, whereas polymerization in the headgroup does not. Covalent linkage of the lipid tails inhibits formation of the gauche rotamers and the cooperativity typically observed as lipid bilayers undergo this phase transition. In some instances polymerizable amphiphiles clearly mimic natural lipids, e.g., phosphatidylcholine (PC)

Scheme 1



and phosphatidylethanolamine (PE), whereas others are more similar to synthetic surfactants, e.g., quaternary ammonium salts (representative examples are shown in Figure 1). Synthetic routes to polymerizable PC and quaternary ammonium lipids were reported as early as 1980, due in part to the commercial availability of synthetic intermediates. On the other hand the lack of corresponding intermediates for PE retarded the use of polymerizable PE until a chemical synthesis was reported.[16]

A variety of polymerizable groups have been successfully employed since the first reports in 1980. The following are the most commonly cited: styryl, diacetylenyl, dienoyl, sorbyl, methacryloyl, acryloyl, and lipoyl (Figure 2). All, but the diacetylenyl group, can be polymerized in the more fluid liquid-crystalline phase (L_α). In contrast, diacetylenic amphiphiles are only polymerized efficiently in the solid-analogous phase (L_β)[17] or in other solidlike assemblies, e.g., tubules,[18] and the condensed phase of Langmuir monolayers.[19] For more information on polydiacetylene vesicles see the recent review by Charych and co-workers.[20] In many instances assemblies composed of lipid diacetylenes could only be partially polymerized due to the topotactic nature of the reaction. This contrasts with the other reactive amphiphiles that may be converted to polymer in high yield (>90%). Photopolymerization is especially effective for diacetylenyl, styryl, dienoyl, and sorbyl amphiphiles, whereas thermally sensitive radical initiators are frequently used for the styryl, dienoyl, sorbyl, acryloyl, and methacryloyl compounds. Redox initiators may also be employed for these monomers plus the lipoyl compounds.[21]

It is well-known that the polymer size and distribution is of prime importance in polymer applications. Therefore, it is crucial to understand the important variables that influence the polymerization process in organized media such as lamellar phases. Of course, some of the same principles which govern polymerizations in isotropic media also apply to the two-dimensional environment of lipid bilayers. Monosubstituted lipids form linear polymers, and bissubstituted lipids, which contain a polymerizable group in each lipid tail, can yield cross-linked polymer networks (Figure 3).[22,23] The polymer size (degree of polymerization, X_n) is sensitive to the relative stability of the propagating species in both bilayer and isotropic polymerizations.[24] Quite large X_n values (10^3−

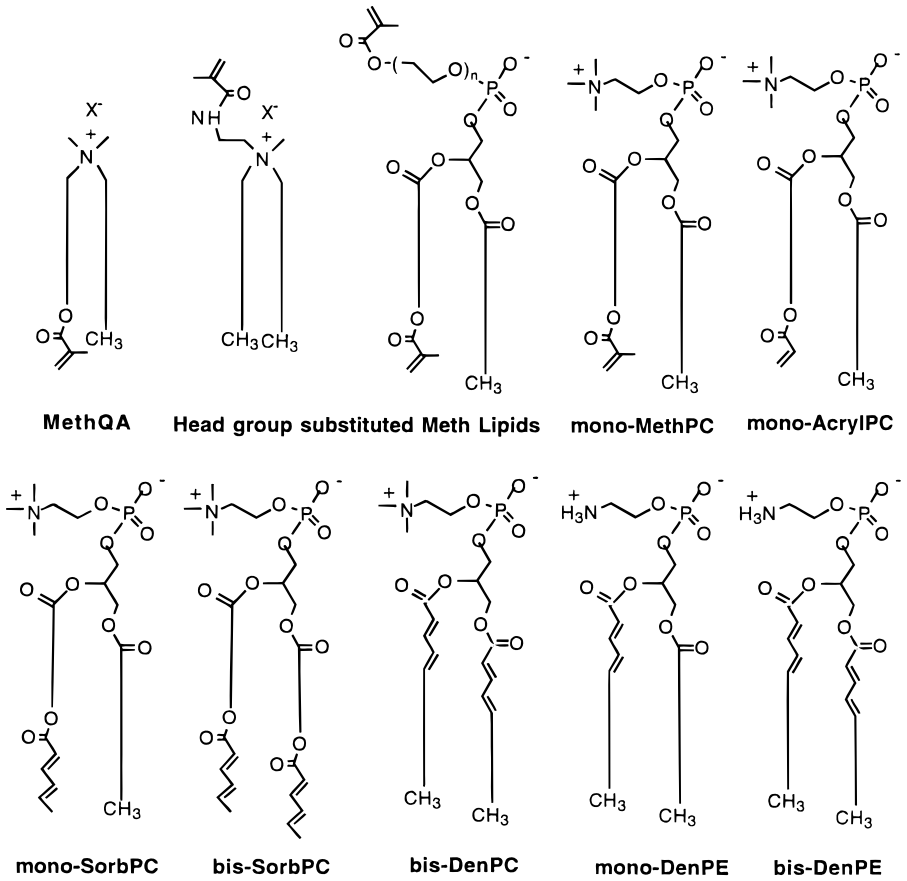


FIGURE 1. Structures of selected polymerizable lipids, where the polymerizable groups are designated Meth (methacryl), Acryl (acryloyl), Sorb (sorbyl), and Den (dienoyl). The lipid classes are quaternary ammonium (QA), phosphatidylcholine (PC), and phosphatidylethanolamine (PE). The fatty acid chain lengths may vary from 12 to over 20 atoms.

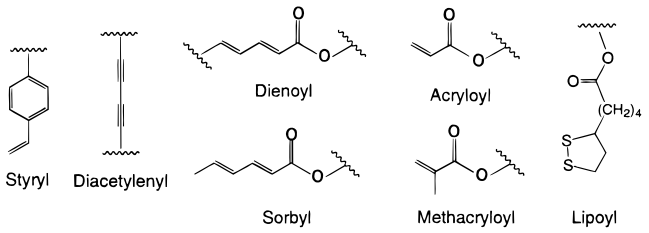


FIGURE 2. Examples of polymerizable groups which have been incorporated into polymerizable amphiphiles.

10^4) have been reported.[25,26] On the other hand, photopolymerization with short wavelength UV light can lead to polymer chain degradation in competition with chain growth.[26] Systematic characterization of the radical chain polymerization of bilayers of mono-AcrylPC (as well as mono-SorbPC) found that at high conversion the X_n was proportional to $[M]^2$ and $[I]^{-1}$, where [M] and [I] are the initial monomer and initiator concentrations, respectively.[24,25] This dependence indicates that radical polymerizations in bilayers do not terminate by bimolecular chain coupling or disproportionation; rather termination is more likely to involve coupling of an initiator radical with the reactive polymer terminus, i.e., primary termination. Analysis of the kinetic behavior of mono-AcrylPC polymerizations supports this hypothesis.[27] Either the translational mobility and/or the segmental mobility of the growing polymer chains may be limited by the

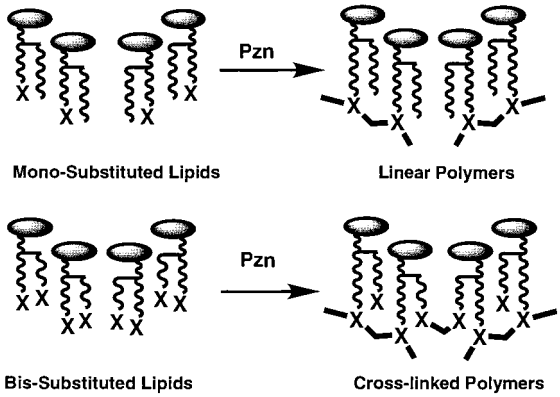


FIGURE 3. Schematic representation of the linear or cross-linked lipid polymers formed by polymerization (Pzn) of either monosubstituted or bissubstituted lipids, respectively.

constrained environment of the bilayer, which tends to increase the lifetime of the radical chain end. In other words, the lamellar assembly effectively constrains termination relative to propagation.

Linear polymerizations cause modest changes in bilayer properties, whereas cross-linking polymerizations can significantly decrease the membrane bilayer fluidity and permeability. Lateral diffusion of bilayer components is substantially retarded by polymerization of bissubstituted lipids.[28] Polymerization of bilayers decreases the bilayer permeability to encapsulated aqueous markers. The

formation of linear polymers in each leaflet of the bilayer reduces the permeability by factors of 2−5, whereas the formation of a cross-linked polymer network in the bilayer leaflets can decrease the permeability by at least 2 orders of magnitude.[29,30] The polymerization-induced decrease in membrane permeability is associated with a parallel increase in the chemical stability of vesicles, which is observed by resistance to dissolution by either surfactants or organic solvents;[22,23] e.g., cross-linked vesicles prepared from a bis-DenPC were not dissolved by excess Triton X-100.[31] Cross-linked vesicles of bis-SorbPC or bis-AcrylPC are also not disrupted by surfactants.[23] These data suggest that a cross-linked bilayer vesicle is composed of a single cross-linked polymer network in each monolayer half of the bilayer. Whether or not the two monolayers are linked together during the polymerization process has not been rigorously examined. The fact that cross-linked vesicles maintain their size and shape even in excess surfactant indicates a two-dimensionally constrained polymerization can yield stable three-dimensional objects. It will be interesting to see if the molecularly smooth surfaces of these vesicles prove useful for chemical and biological modification.

Cross-linking is associated with a gel point, i.e., the point of polymer insolubility. However, in the case of vesicles, the cross-linked polymer is confined to the bilayer, whereas the aqueous medium supporting the vesicles is still fluid. Consequently quite large polymers and polymer networks can be formed without changing the overall sample fluidity. If cross-linked vesicles are freeze-dried, then the resulting dry polymer exhibits general insolubility in organic solvents, including hexafluoro-2-propanol (HFIP).[23] The incorporation of a bifunctional compound within a bilayer of a monosubstituted lipid can cause cross-linking. The comparative effectiveness of bifunctional compounds as cross-linkers can be determined from the minimum mole fraction of the bifunctional compound needed to produce cross-lined bilayers. Bissubstituted lipids, e.g., bis-SorbPC or bis-DenPC, are useful bilayer cross-linkers. Small-angle X-ray diffraction and NMR data indicate that the glycerol backbone is nearly perpendicular to the bilayer plane.[32] Therefore, two equal length acyl chains penetrate unequally into the bilayer, causing a positional inequivalence of the reactive groups. Approximately 30 mol % of the lipids in bilayer vesicles composed of mono- and bis-SorbPCs must be bis-SorbPC in order to produce cross-linked vesicles.[23] Vesicles composed of the more reactive AcrylPCs required a similar fraction of bislipids to be effectively cross-linked. However, if the reactive group is closer to the glycerol backbone, i.e., DenPC, then only 10 mol % of the bislipid was necessary to cross-link the vesicles.[33] This difference in cross-linking effectiveness may be due to the greater conformational freedom of reactive groups located at the end of the lipid tail, rather than near the glycerol backbone of the lipid. Processes that compete with the cross-linking, such as macrocyclization, are favored by greater freedom of motion.

The above studies indicate that the distinctive nature of polymerizations in lyotropic mesophases is due in part

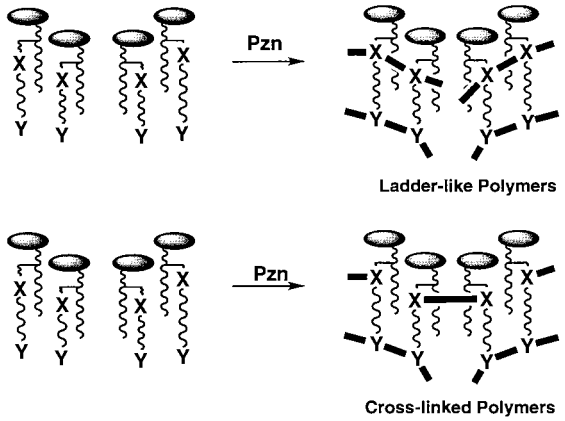


FIGURE 4. Schematic representation of the polymers formed from dependent (ladderlike) and independent (cross-linked) polymerization (Pzn) paths of two reactive groups tethered via an aliphatic chain.

to the anisotropic orientation and restricted motion of monomers. Other studies have highlighted the differences between polymerization reactions in organized and isotropic media. The inherent orientational order in smectic liquid crystals was utilized to create two-dimensional polymers.[34] Analysis of rates of polymerization in smectic mesophases shows significant effects on the rates of propagation and termination compared to isotropic systems.[35,36] Quite recent research shows that lyotropic mesophases of a double-diene PC offer the possibility of selective polymerization.[37,38] Because the two diene groups are located in regions of different polarity, it is possible to perform either simultaneous or selective and sequential polymerizations. The selective polymerization of either group can be used in tandem for sequential polymerization. If the reaction path of the second group to react is uncorrelated with the reaction path of the first group, then cross-linking should occur (Figure 4). However, if the second reaction occurs preferentially with an adjoining group in a repeat unit of the polymer formed in the first reaction, then a ladderlike polymer is possible. These two possibilities were distinquished for the double-diene PC by surfactant treatment of the polymerized vesicles. Sequential polymerization was performed by either (1) selective photopolymerization of the dienoyl, followed by redox polymerization of the diene, or (2) selective AIBN polymerization of the diene group, followed by redox polymerization of the dienoyl (Figure 5). Persulfate production of hydroxyl radicals was used for simultaneous polymerization. Each of the three modes of polymerization gave polymerized vesicles that were disrupted by Triton X-100, showing the lack of cross-linking. These results indicate that both groups preferentially react with the same neighbor lipid. An alternative interpretation that their reactions occur in nonoverlapping domains of the bilayer was excluded by the observed high conversion to polymer. Since the preferred mode of polymerization of the double-diene PC may be a consequence of the short spacer link between the two dienes, we are examining the effect of the spacer length on the competitive reaction paths in lyotropic mesophases.

Cross-linking polymerization of bilayers composed of a bissubstituted lipid, e.g., bis-SorbPC, in the presence of

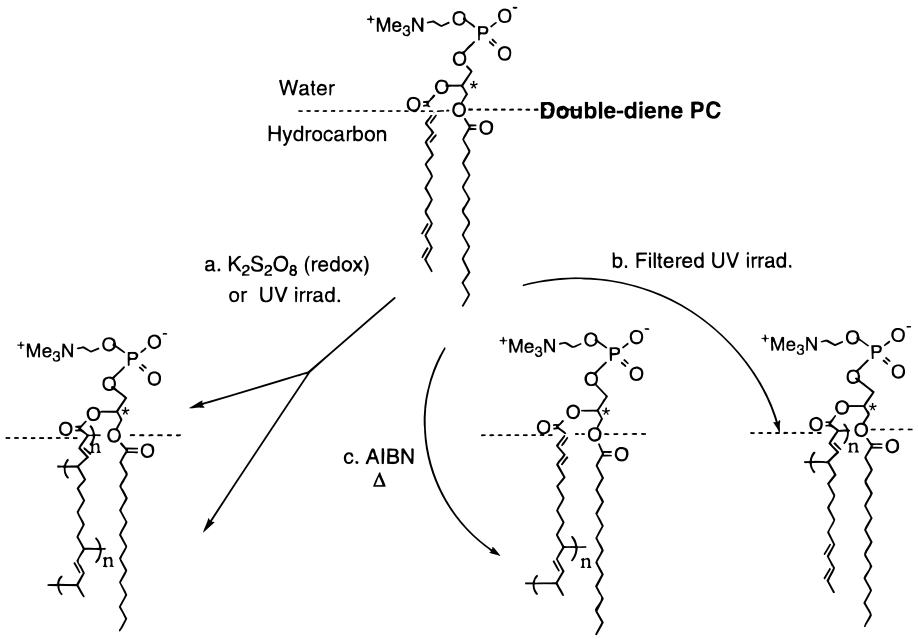


FIGURE 5. Methods of selective and simultaneous polymerization of hydrated double-diene PC bilayers.

a nonreactive colipid can "lock-in" preexisting lipid domains or create lipid domains from random lipid mixtures, depending on whether the original unpolymerized bilayer mixture is heterogeneous or homogeneous, respectively. Numerous studies that demonstrate both circumstances were recently reviewed.[39] Cross-linking polymerization has been usefully employed to reorganize the lateral distribution of surface-associated chromophores,[40] to create skeletonized vesicles composed of domains of cross-linked poly(lipid) and voids caused by surfactant, solvent, or enzymatic lysis of nonpolymerized domains,[8,41] or to "destabilize" bilayer vesicles by concentration of so-called polymorphic lipids into regions that can readily form precursors to the nonlamellar phase.[42] The latter case can be employed to release reagents from vesicles or to deliver them to other bilayer-bounded structures via vesicle fusion.[43] The cross-linking of bis-SorbPC in the presence of a PE, e.g., dioleoylPE, produces enriched domains of PE. Vesicles composed of homogeneous mixtures of PE and PC lipids are usually separated from neighboring bilayers by the strong hydration of the PC. The photoinduced formation of enriched domains of the less strongly hydrated PE reduces the repulsive hydration force and permits the close approach of the PE-rich regions of the bilayer. Contact between opposing lamellae enriched in PE leads to the formation of nonlamellar intermediate structures that were detected by ^{31}P NMR and X-ray diffraction.[44] Moreover, the photoinduced destabilization of bilayer vesicles was manifested by the release of the encapsulated aqueous contents and/or vesicle fusion with other bilayer structures. In the latter case photopolymerization of bis-SorbPC reduced the critical fusion temperature by 20 °C, thereby permitting the design of vesicles that are stable at 37 °C prior to photolysis and destabilized after photopolymerization.[45] These results coupled with the well-developed methods for controlling the spatial and temporal delivery of light endow the photoinduced destabilization of vesicles with a valuable capability to regulate the release of chemical and/or biological reagents. Furthermore, this ongoing research provided a bridge to studies of the bicontinuous cubic phases, which can form from the fusion intermediates, and to methods to polymerize these and other nonlamellar phases.

Polymerization of Nonlamellar Phases of Hydrated Lipids

The initial goal of the research on nonlamellar phases was the demonstration of a useful polymerization strategy for the stabilization of these three-dimensional lyotropic liquid crystals in order to expand their rather limited useful temperature and concentration ranges. The design of suitable polymerizable lipids was crucial to the success of the research. Certain characteristics of nonlamellar phases limit the lipid structures that can be employed for polymerizable lipids. Since both the Q_{II} and H_{II} phases are disordered and the lipids laterally diffuse even more rapidly than in the L_α phase, it is unlikely that diacetylenic lipids would be suitable. Moreover, it was desirable to be able to perform the polymerizations at a variety of temperatures as dictated by the phase behavior of the lipid, thereby limiting the use of thermal initiation chemistries. Consequently reactive groups, such as dienes, that may be polymerized by light or redox chemistries have received the most attention. Unlike lamellar phase polymerizations, the location of the reactive group in the lipid and the assembly requires careful consideration. The placement of the group on either or both of the lipid tails near the lipid backbone, i.e., the glycerol unit in the case of phospholipids, appeared to be the most promising because covalent linkage of lipids near the backbone should have less effect on the important forces that act at the headgroup and tails of the lipids. Furthermore, the

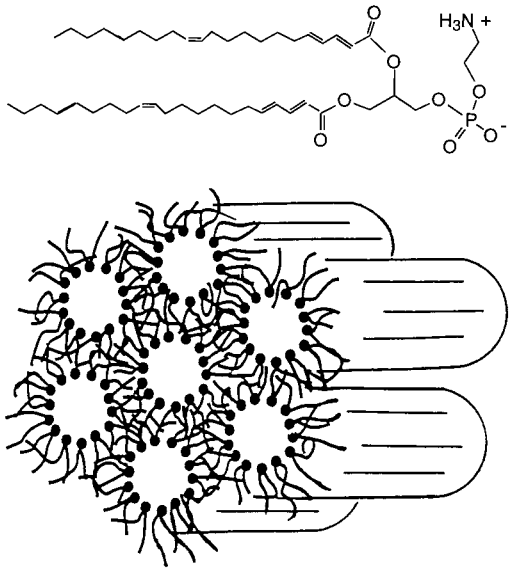


FIGURE 6. A polymerizable phosphatidylethanolamine used to form and polymerize the H_{II} phase (bottom). Note the disordered tails of the lipids and the headgroups surrounding a water channel.

use of a diene group conjugated with the acyl chain carbonyl does not interfere with the biocompatibility of the lipid−water interface. These factors were used to design suitable lipids for the polymerization of nonlamellar phases. The selection of lipid structures also included consideration of the spontaneous radius of curvature, R_0,[6,7] which can be used to predict the mesomorphic behavior of lipids or lipid mixtures. Lipids with small absolute R_0 values require less thermal energy to undergo L_α/H_{II} transitions, whereas those with large absolute R_0 values form stable L_α phases even at high temperatures. Hydrated lipids with intermediate absolute R_0 values are likely to form Q_{II} phases at moderate temperatures.[46,47] Unsaturated or chain-substituted PE lipids are the best known compounds with small R_0 values,[48] whereas those with intermediate values include certain PC/PE mixtures, *N*-methylated PEs, and monoacylglycerols (MAG).

The most commonly studied nonlamellar lipid phase is the H_{II} phase, which can be considered as aqueous columns arranged in a hexagonal pattern (Figure 6). Each water channel is encased within a monolayer of lipid. The polar headgroups are well-ordered at the water interface, whereas the lipid tails are highly disordered to fill the volume between the columns of water. A particularly interesting characteristic of the H_{II} phase is the decrease in the unit cell dimension of the hexagonal lattice as the sample temperature is increased. Reduction in the radius of the water channel accounts for most of the change in this lattice vector.[49] It appeared that a successful polymerization strategy could lock-in the column diameter, rendering the unit cell of the H_{II} phase insensitive to changes in temperature variation. Consequently a polymerizable PE with dienoyl groups in each lipid tail was prepared and used to form the H_{II} phase, which was then cross-linked around and along the water cores.[12] The hydrated PE (1:1 by weight) formed the H_{II} phase over an extended temperature range (near 0 °C to at least 80 °C).

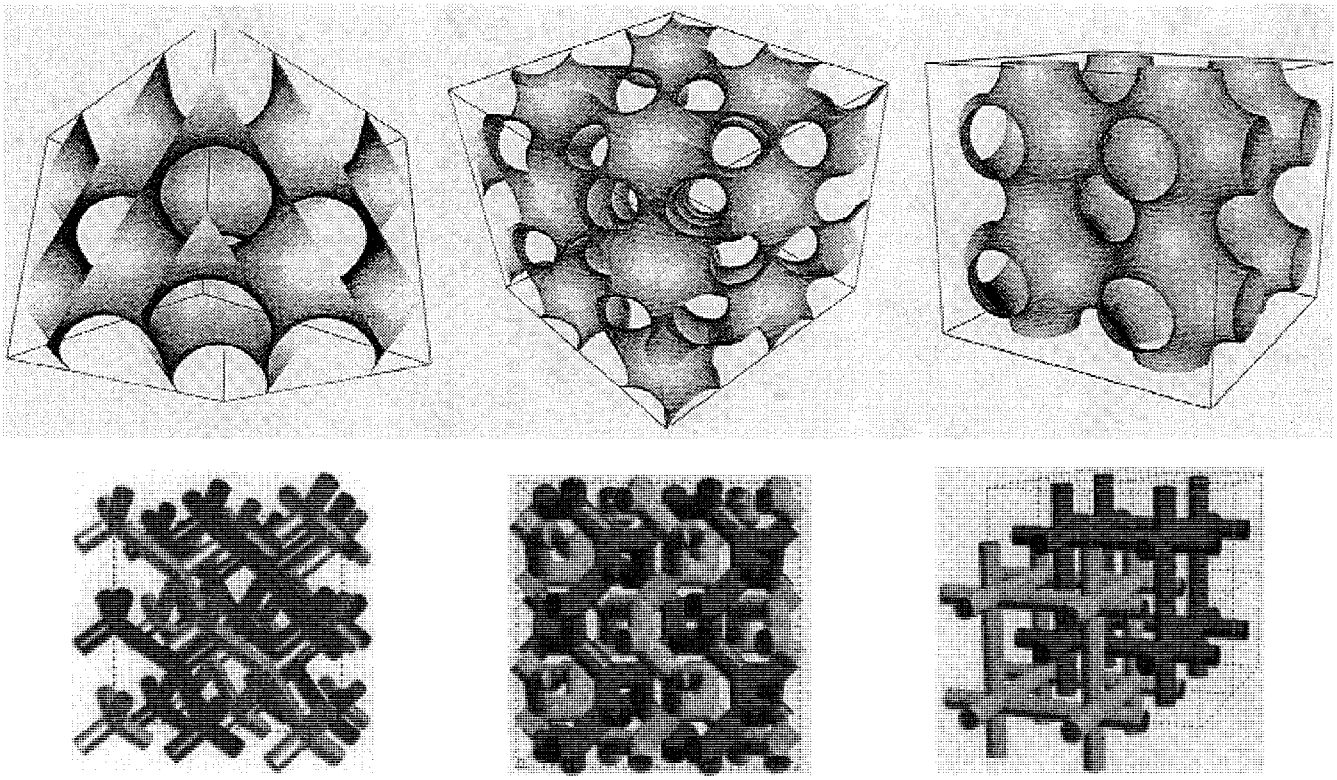


FIGURE 7. Top: Mathematically generated minimal surfaces that represent three bicontinuous cubic morphologies associated with hydrated lipids. These surfaces correspond to the lipid portion of the respective phases (space groups from left to right, $Pn\bar{3}m$, $Ia\bar{3}d$, and $Im\bar{3}m$). Bottom: Representation of the three-dimensional networks that are complementary to the above surfaces. These labyrinths correspond to the water portion of the respective phases obtained from hydrated lipids.

Polymerization to high conversion was accomplished with redox initiators. Characterization of the polymerized material by X-ray diffraction and ^{31}P NMR indicated that it remained in a hexagonal phase. As predicted, temperature cycling of the polymerized H_{II} phase did not alter the dimensions of the unit cell. Moreover, this H_{II} phase polymerization proceeded by cross-linking of the individual tubes, because the polymerized sample could be dispersed in volatile organic solvents and then spread as a monolayer of the polymerized unit cells, i.e., tubes of poly(lipid) each surrounding a water core.

The stabilization of cubic phases, which are bicontinuous with respect to the polar (aqueous) and nonpolar (lipid) regions, is especially interesting because these phases are organic analogues of zeolites (Figure 7). Polymerization of the lipid domains should yield materials with interpenetrating water channels, whose surfaces may be functionalized with chemical or biological labels or reagents in a suitable manner for diagnostics, separations, and conversions. The size of the aqueous channels is a function of the lipids and can range from ca. 4 to 20 nm in diameter, large enough to accommodate certain water soluble macromolecules. In 1995, Lee et al. reported the formation and polymerization of a Q_{II} phase composed of a mono-DenPE and a bis-DenPC in a 3:1 molar ratio.[11] Both X-ray diffraction and ^{31}P NMR spectroscopy were used to partially characterize the phase behavior of the lipid mixture, and then radical chain methods were used to cross-link the Q_{II} phase. Electron microscopy of the polymerized samples revealed the water channels were 6 $\pm$ 1 nm in diameter. More recently, Srisiri et al. showed that a polymerizable monoacylglycerol combined with the corresponding diacylglycerol in a 9:1 molar ratio forms an optically clear Q_{II} phase.[13] Phase investigation using cross-polarized light, ^{2}H NMR spectroscopy, and X-ray diffraction found the well-defined cubic phase exists from at least 5 to 45 °C. The X-ray diffraction pattern corresponded to a cubic phase with $Ia\bar{3}d$ symmetry and a unit cell size of 13 nm at 25 °C. Polymerization of the reactive amphiphiles increased the stability of the assembly with retention of the Q_{II} phase. Water diffusion inside the aqueous 3-D channel network remained the same both before and after polymerization, indicating that polymerization did not alter the structure of the interpenetrating water channels in the cubic phase.[13]

The polymerization of suitably designed lipids in both the H_{II} and Q_{II} phases has proven to be an effective means to enhance the stability of these lyotropic liquid crystals. This early research provides a basis for the design of other chemical strategies to stabilize nonlamellar phases. The mesoporous nature of the polymerized nonlamellar phases suggests their use for the incorporation of synthetic or biological molecules at binding sites localized along the water channels. There is also considerable interest in the prospect of using Q_{II} phases as biocompatible encapsulating and controlled release media. In addition stabilized nonlamellar phases could serve as templates for the preparation of inorganic−organic composites. Continued progress in this research will require interdisciplinary contributions of biological, polymer, and surface sciences.

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